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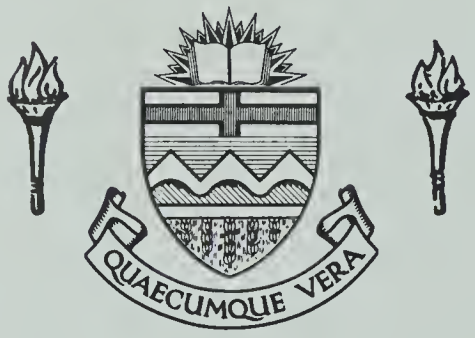

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STUDIES ON THE UPTAKE AND DISTRIBUTION OF CERIUM-141 IN THE RAT

by

ALEXANDER SHYSH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled: "STUDIES ON THE UPTAKE AND DISTRIBUTION OF CERIUM-141 IN THE RAT", submitted by Alexander Shysh, in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The uptake and distribution of cerium-141 was studied in various organs of the rat after intravenous administration. Twenty-four hours later, almost the entire injected amount was distributed throughout the liver, spleen, kidneys, skeleton and excreta. Levels of the isotope were highest in the liver and skeleton. Cerium-141 was rapidly concentrated in the liver with a biological half-life of about 15 days in this organ. Uptake by the bones was more gradual with a prolonged retention. Excretion of cerium-141 was almost entirely via the fecal route. Young animals retained higher amounts of the isotope in the liver and skeleton than older mature rats.

Autoradiographs of long bones indicated that cerium-141 was deposited in the zones of active growth beneath the epiphyses, on endosteal and periosteal surfaces, and perivascularly throughout compact bone. Dietary deficiencies in vitamin A, calcium, phosphorous, or a rachitogenic diet, all resulted in an elevated uptake of radiocerium by bone. Calcium disodium versenate was ineffective in mobilizing skeletal deposits of the isotope. Lack of vitamin A prolonged the hepatic retention of radiocerium. The probable mechanisms by which these dietary factors may have increased radiocerium deposition are discussed.

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INTRODUCTION

In spite of its relative natural abundance and early discovery, practical interest in cerium was not awakened until the atomic era. Discovery of economical methods of extraction and purification (1,2) rapidly prompted industrial utilization of this metal and introduced aspects of chemical toxicity and industrial hygiene. However, it was the prominence of cerium isotopes in high yields among fission products that stimulated research into the pharmacology, radiotoxicity and metabolic pathways of cerium in biological systems (3,4,5).

Present knowledge of the behavior of cerium and the rest of the rare earths in living systems has been obtained almost exclusively from the use of the radioactive isotopes of these elements.

Several workers have investigated the distribution of cerium in various laboratory animals (6,7,8,9). Shortly after parenteral administration of cerium, a very high and rapid uptake by the liver was noted with over 50 per cent of the dose accumulating in this organ and about half of this amount being excreted in 15 days. While deposition in the skeleton was somewhat slower and accounted for about 20 per cent of the injected dose, elimination of the radiometal from the skeleton was much slower than from the liver. Because of prolonged retention of large amounts of cerium by bone tissue, the skeleton has been considered as the critical or target organ for this element (10,11).

It was the purpose of this investigation to study the

cerium levels in various soft tissues of the rat following cerium-141 administration. However, results indicated that brain, cardiac muscle, skeletal muscle, lungs, seminal vesicles, prostate, thyroids and testes did not appreciably concentrate this isotope. Almost the entire administered activity was distributed among the liver, skeleton, spleen, kidney and excreta. Since the skeleton was indicated to be the target organ of greatest biological significance, it was decided to localize the site of radioactive cerium deposition in bones by autoradiography and then to study the effects of well-established bone development factors upon the uptake, distribution and excretion of cerium in the rat. The parameters investigated were, age of the animals, and the following deficiency diets: rachitogenic diet, calcium-low diet, phosphorous-low diet and vitamin A-deficient diet. The results of these studies further prompted a preliminary investigation into the efficacy of calcium versenate as a chelating agent for removal of radiocerium after deposition.

SURVEY OF THE LITERATURE

1. Historical

Cerium, atomic number 58, is the most abundant element in the group known as the Lanthanide series, commonly referred to as the Rare Earths (8). This element was discovered in 1814 by the Swedish chemists Berzelius, Kalaproth and Hisinger, and named after the newly sighted asteroid Ceres. Mineral deposits of monazite, cerite, allanite and bastnasite containing cerium along with other lanthanides occur in Scandinavia, Brazil, India, Ural Mountains and the United States of America (1,8,10). Cerium ranks 28th in abundance among the naturally occurring elements and is more plentiful in the earth's crust than such well-known metals as cobalt, lead, tin, uranium, gold, mercury or silver (1,8). However, cerium lacked practical interest in the past partly because of the great difficulty of its preparation in a pure form, and partly due to the lack of any known metabolic role of cerium in living organisms (12). The development in 1947 of an efficient isolation and purification process for the rare earths employing ion exchange resins stimulated industrial use of cerium (1,2,13,14,15). Presently, cerium is used commercially in metallic alloys, ceramics, glass and optics manufacture, catalysts, lighter flints and pyrotechnics (1,2,16). However, the current biological accent on cerium is a result of modern nuclear technology.

2. Cerium Isotopes as Fission Products

After fission of uranium-235, about 200 radioactive nuclides of some 35 light elements are formed (10). Thirteen

radioactive isotopes of cerium appear among the fission products, and are predominant up to three years after fission (3,4,10). One of the possible routes of uranium-235 fission was shown to produce cerium-141 in the following chain (4):

$$_{92}U^{235} + {}_{0}n^{1} \text{ ---------- } {}_{56}Ba^{141} + {}_{36}Kr^{89} + 6\,{}_{0}n^{1}$$

$$t^{1/2}=18 \text{ min.} \quad \downarrow \beta, \gamma$$

$${}_{57}La^{141}$$

$$t^{1/2}=3.7 \text{ hr.} \quad \downarrow \beta, \gamma$$

$${}_{58}Ce^{141}$$

$$t^{1/2}=28 \text{ days} \quad \downarrow \beta, \gamma$$

$${}_{59}Pr^{141} \quad \text{(stable)}$$

The main isotopes of cerium produced during fission are:

Isotope	$t^{1/2}$	Mode of Decay	Appearance after Fission
Cerium-141	32 days	γ ,β	Prominent up to 1 yr.
Cerium-143	33 hours	γ ,β	Prominent up to 1 mo.
Cerium-144	290 days	γ ,β	Prominent from 1 to 3 years

These three isotopes comprise approximately 16 per cent of the total fission yield (8). Cerium-144 occurs together with its radioactive daughter, praseodymium-144, which has a radioactive half-life of 17.5 minutes and emits powerful beta particles having maximum energies of 2.98 and 2.3 Mev (17). Cerium-144 and praseodymium-144 together are extremely important

in the handling and reprocessing of irradiated fuels, accounting for three-quarters of the beta and one-third of the gamma activity given off by fission wastes during the first year of decay. Cerium-144 was the energy source in a nuclear battery developed for satellite power (10). Recent reports from Japan have identified considerable levels of radioactive cerium isotopes in fallout from Russian (1961), and Chinese (1964, 1966) nuclear explosions (5,18,19).

The interest created by the availability of commercial quantities of cerium and its predominance in uranium fission products introduced such familiar problems as chemical toxicity, industrial hygiene, radiotoxicity and disposal. These recent developments illustrate the importance of knowledge concerning the behavior of cerium in living systems, and its influence on metabolic processes.

3. Biological Occurrence and Response

a. Soils and Plants

Because cerium is relatively abundant in the earth's crust (1,8), a lack of this element in soils is not a likely cause for absence of cerium in plant tissue. It has been suggested that such absence in plants is due to a discrimination against absorption of cerium by root systems (20). The behavior of rare earth metals, especially cerium, in soils and accumulation in crops has been an urgent problem in the study of fallout from fission products.

Upon surface application of cerium-144 to a variety of soils, 85 per cent of the isotope remained in the upper 5 cm. layer even after exposure for two years, indicating the

firmness of the cerium binding by soil (21). Soil experiments showed that virtually no cerium-144 was taken up from different soils by barley, bean, carrot, lettuce, and radish plants. In contrast, these same experiments showed that strontium-90 was readily taken up, and concentrated in leaf tissue (22). In general, plants tend to provide an efficient barrier against transfer of rare earths in fission products from soil to animals (20).

b. Marine Systems

In a prolonged study on radioactive cerium contamination of a complex fresh water community, Fontaine (23,24) found that cerium-144 was rapidly extracted from the water by fixation on all solids and living organisms. Green algae and water fleas were implicated in particular. Eighteen days after introduction, the concentration of radiocerium in the algae, water fleas, mollusc viscera, and the contents of digestive tracts of fish varied between 20 to 300 times that of the initial radioactivity of the water. Palkorpov (25) determined the accumulation coefficients (ratio of concentration in the organism to that of the aqueous medium) for a series of radioisotopes and 21 representatives of sea algae, grasses, coelenterates, crustacea, and molluscs. Specific concentrations of cerium-144 were shown to be present in various species of algae, eelgrass, actinia, crabs, molluscs and mussels. Cerium-144 was also detected in marsh clams at Ibaraki, Japan (26). Since radioactive contamination of the marsh beds originated from fallout, it was assumed that the clams absorbed the radiocerium from the mud bottom. Interestingly, the levels

of cerium-144 in the clams were much higher than those of strontium-90.

Rudakov (27) illustrated that when fingerling trout and carp were immersed in a cerium-144 solution for one hour, the radiocerium was rapidly absorbed from the water with initial accumulation in the gills and liver, and in bones after a period of a month.

c. Micro-organisms

Investigations into the uptake of radiocerium and other fission products by various bacteria, yeasts, and moulds showed significant uptakes of cerium in all cases where adequate growth was observed (28). With few exceptions, the uptake of radiocerium was considerably higher than that of mixed fission products. However, there has been no evidence to suggest that accumulations of rare earths during bacterial growth involves any essential relationships (29). The following groups of micro-organisms and the individual species of each group have been noted to concentrate radiocerium (17):

Bacteria ----------E. coli

Streptomyces --------Streptomyces flavovirens

Yeasts --------------Saccharomyces cerevisae

Moulds --------------Members of the mucorales:
(A. glauca, A. niger, R. nigricans)

The accumulation of large amounts of cerium by E. coli and other organisms of common occurrence in the soil and sewage may be of possible significance when radioactive wastes are buried or disposed through sewage systems.

Use of higher concentrations of cerium than those of

the carrier-free radioactive forms employed in the tracer studies indicated that the salts of cerium may be bacteriostatic (30). In concentrations varying from 0.0004 to 0.0012 M, the chloride and nitrate salts of cerium prevented growth of some 39 species of bacteria. Salts of cerium were found to be more toxic for bacteria than for fungi.

d. Mammals

Cerium has no known functions in the mammalian organism (12). Examination of normal human tissue by neutron activation analysis combined with ion-exchange separation failed to show any significant, naturally occurring, concentrations of cerium (31,32). However, the presence of cerium-141 and cerium-144 has been detected at low levels in some animal bone (26) as well as in lung tissue and pulmonary lymph nodes from man (33,34).

4. Absorption of Cerium

From a practical viewpoint, the most important modes of entry of a radiometal into the body are through the gastrointestinal tract, aerogenous route, wound contamination or percutaneous absorption (35). Investigation by a succession of workers utilizing tracer methods have indicated that the distribution of cerium was greatly dependent upon the chemical form and route of administration. A number of reports have indicated that there was essentially no absorption of cerium from the gastrointestinal tract. Durbin (7) administered cerium citrate orally to rats and found that the absorption of the element was less than 0.1 per cent of the administered dose after four days. Similar results for a variety of small animals were reported by other investigators (6,7,8).

Although grazing animals may ingest large amounts of cerium-144 and other radionuclides as contaminants of foliage in the form of dust and dirt, very little cerium was found to be absorbed from the gastrointestinal tract (37,38). Gastrointestinal absorption in ewes did not exceed 0.03 per cent of an ingested amount of cerium-144 (36). Only 0.01 per cent of an administered dose of cerium-144 was recovered in milk in a dairy cow after oral administration (38).

During nuclear fallout, radionuclides which appear in the air could be introduced into the body by inhalation. Results have indicated that levels of radiometals after inhalation may correspond rather well to those produced by intravenous injection (39). An uptake of cerium-144 in the pulmonary lymph nodes after inhalation has been observed in man (33,34). Animal experiments involving radiocerium inhalation indicated an initial high lung concentration followed by liver and skeletal uptake (40,41,42). Following aerosol administration, an estimated biological half-life of 50 days in the pulmonary system was reported for the insoluble cerium-144 oxide (10,20), while that of the soluble cerium-144 chloride was somewhat shorter (39). Considerable deposition in the liver and skeleton following inhalation indicated that cerium may have passed from lung to blood directly, and possibly by way of the lymph (20).

Absorption of rare earths from sites of subcutaneous, intramuscular, or intraperitoneal injection was shown to vary greatly with the chemical state of the element. Injection of a readily ionizing form such as the chloride or nitrate salt

resulted in slow and incomplete absorption, whereas low ionized complexes such as cerium versenate were absorbed more rapidly (43). Four days after an intramuscular injection of cerium citrate, 94 per cent of the administered dose was absorbed (7).

In the majority of metabolic studies appearing in the literature, radiocerium had been administered intravenously as a chloride or nitrate salt, and adjusted to a low pH to minimize colloid formation (8).

5. Distribution of Cerium

The blood level of cerium in rats following intravenous injection was found to decrease rapidly to 1 per cent of the administered dose per ml. of serum within one hour, and to less than 0.02 per cent per ml. of serum after one day (7, 8). In the blood, cerium was not found in the erythrocytes and thrombocytes, but partially incorporated into the nucleic acids of the leukocytes and bound to protein constituents (8). Complex formation of cerium with serum albumin indicated a possible means of transport in the blood (44).

Investigations carried out on mice, rats and guinea pigs illustrated that the rapid fall in cerium blood levels coincided with a high concentration in the liver (7,8). Within several hours after administration, the liver contained over 70 per cent of the dosage and decreased gradually to approximately 50 per cent after four days (8). Cerium appeared to be strongly bound to the parenchyma of the liver, initially distributed around the vascular portions and later associated with Kupffer cells (8,20). Magnusson (8), in his

investigation of the subcellular distribution of cerium-144 in liver cells, separated liver homogenates into four fractions by differential centrifugation: nuclear, mitochondrial, microsomal, and the remaining supernatant which he considered to be cytoplasmic matrix. The isotope could be detected in all fractions at various times after administration. The initial high amounts which existed in the supernatant and gradually dropped, indicated that cerium was probably first deposited in the cytoplasmic matrix of the liver cell and then transported to various cell constituents. In both male and female rats, the nuclear fractions contained the lowest amounts of the nuclide. In females, the level of cerium was highest in the microsomal fraction, while in males the concentration predominated in the cytoplasmic matrix. A similar investigation by Catsch *et al.* (45) gave essentially the same results.

Biologically, the most important deposition site for cerium, as well as the rest of the rare earths, is the skeleton. Durbin (7) showed that one-third of the radioactive cerium deposited in bone was rather labile with a half-life of about 15 days, but that the remaining two-thirds were firmly fixed and remained at the same level for the duration of the experiment (256 days). Biological turnover of cerium in bone tissue is extremely slow. A biological half-life of 1,500 days in rat skeleton is currently listed for cerium-144 (10). However, a recent study of long-term *in vivo* retention of cerium-144 in beagles, employing whole body counting, indicated that the biological half-life may be as long as 10

years (46). Autoradiographs of long bones have demonstrated the deposition of cerium isotopes on the endosteal and periosteal surfaces and in the vicinity of small blood vessels in the epiphyseal region and throughout cortical bone (6,7,9,47, 48). Histologic and autoradiographic localization indicated that radiocerium was deposited mainly on bone mineral wherever it came in contact with blood, while somewhat smaller amounts of the isotope appeared in osteoid tissue (49). In dogs treated with radiocerium, Jowsey, Rowland and Marshall (50) showed that the isotope was only slightly retained by bone protein but deposited primarily on highly mineralized non-growing surfaces. Accumulation of radiocerium in teeth was limited to the enamel and pulp with very little activity appearing in the dentine (9).

The degree of concentration of cerium in the spleen appears to depend largely upon whether the element is present in the blood stream in a colloidal or non-colloidal form. Durbin (7), using cerium in the form of a citrate complex, found a low (less than 0.5 per cent per gm.) uptake by the spleen. Intravenous administration of the rare earths as halides or nitrates resulted in high concentrations in the spleen and other components of the reticuloendothelial system (43). Aeberhardt *et al.* (51), upon adjusting the pH of cerium nitrate solutions below 3 and that of cerium chloride below 4.5, were able to minimize radiocolloid formation and showed a low uptake by the spleen under these conditions.

Within the first hour after injection, the concentration of cerium in the kidneys was second only to that of the

liver (7). Autoradiography of mouse sections, 20 minutes after cerium-144 injection indicated an intense uptake by the kidneys, with radioactivity predominating in the renal cortex (9). However, within three hours, the level in the kidneys dropped to approximately 2 per cent of the administered dose (8), and subsequently decreased to lower levels (7). After removal of the skeleton, liver, spleen, and kidneys, Durbin (7) found that 4 days after administration, the soft tissue balance contained less than 6 per cent of the original dose.

Ewaldsson and Magnusson (9) employing whole body autoradiography of mouse sections, studied the localization of radiocerium in a variety of tissues up to 24 hours after injection. These workers noted that in addition to the liver, skeleton, spleen and kidneys, uptake was also observed in the adrenal cortex, ovaries, superficial gastric mucosa, lungs and mammary glands. Radiocerium was shown to cross the placental barrier readily. The level of activity in the placenta appeared to be much the same as that of the blood. Some accumulation of the isotope by fetal membranes was noted and heavy concentration in the liver and skeleton of the fetuses was evident.

Using mice, Kulikova (52) found that large amounts of radiocerium were transmitted to the embryo via the placenta only in cases where the cerium was administered during pregnancy. If the radiocerium was injected a few days prior to pregnancy, very little was passed into the embryo.

6. Excretion of Cerium

Excretion of cerium was found to be almost entirely fecal. Durbin (7) recovered only 8 per cent of an administered dose in a cumulative urinary sample after 16 days, with no further excretion noted via the urine for 256 days. However, in the same experiments, fecal elimination of the cerium rose steadily, with 30 per cent excreted after 16 days and 60 per cent after 256 days. Magnusson (8) reported urinary levels below one per cent and an autoradiographic study of whole body sections in mice did not illustrate any radioactivity in the urinary bladder (9).

Fecal excretion of radiocerium appears to be due to direct elimination through the gastric mucosa and via the bile. Magnusson (8) found concentrations of radiocerium in the gastric mucosa after intravenous administration, and by examination of intestinal sections concluded that excretion was greatest in the stomach and decreased in the intestine aborally. Confirmation of radiocerium excretion through the gastric mucosa was obtained by the autoradiographic method, which illustrated intense activity in the superficial gastric mucosa in the first hours after injection (9).

Durbin (7,48,49) explained that the large amounts of radiocerium in the feces were due to elimination from the liver via the bile. Magnusson (8) by catheterization of the common bile duct, was able to demonstrate the presence of radiocerium in bile 20 minutes after intravenous injection. Castellino et al. (53), in a similar experiment, showed that no intestinal reabsorption of cerium-144 occurred and that

administration of choleretics produced no significant change in biliary elimination of radiocerium. Electrophoretic separation of the bile indicated that the cerium had probably been bound to the protein portion of the bile (53), although the possibility of binding to bile acids has also been raised (8).

7. Toxicity of Stable and Radioactive Cerium

The toxicity of cerium may be considered under two different aspects: chemical and radioactive. The increased industrial use of cerium has prompted investigations into the chemical toxicity of the element, whereas interest in cerium resulting from fission products has introduced hazards of radiotoxicity.

Early investigators considered cerium to be relatively non-toxic because of poor absorption from the gastrointestinal tract. Oral doses exceeding 2.0 gm./kg. in rats were tolerated and hazards associated with ingestions of stable cerium were those of mucosal injury to the tract (54).

In a toxicity evaluation of rare earth salts, following intraperitoneal administration, Gracia, Garst and Lowry (55) noted that the rare earths precipitated very readily in the physiological pH ranges, probably as phosphates or carbonates and that insoluble complexes were formed with nucleic acids. Thus, when rare earth salts were injected into animals, precipitation occurred at the site of injection and initiated a localized inflammatory process. The $L.D._{50}$ of $CeCl_3$ in mice following intraperitoneal injection was observed to be 350 mg./kg. However, solutions such as cerium citrate,

which formed soluble complexes stable up to pH 8 or higher, when injected into the animals did not precipitate readily. The absorption was therefore enhanced, and inflammatory reactions were minimized. As a result of improved absorption, the toxicity of stable cerium in the form of citrate was increased such that the $L.D._{50}$ was only 146 mg./kg. after intraperitoneal injection (55).

In their toxicological investigation, Kyker and Cress (56) reported a lethal dose of 50 to 60 mg./kg. for $CeCl_3$ after a single intravenous injection in the rat. An intravenous dose of 3.5 mg./kg. was found to produce mortality in 11 per cent of the test animals.

Steffee (57) was interested in the toxicity of stable rare earth compounds because of possible therapeutic value of radioisotopic forms in treatment of malignant peritoneal or pleural effusions. As previously mentioned, readily ionizable salts of the rare earths were shown to be only slightly absorbed from sites of intraperitoneal injections (43). Steffee (57) reported that intraperitoneal administration of stable cerium chloride at dosage levels of 100 mg./kg. to rats resulted in an acute peritoneal reaction which subsequently developed into a true granulomatous peritonitis. Gross pathologic examination of animals following intraperitoneal administration of stable cerium, revealed a loose caseous precipitate of the rare earth in the abdominal cavity along with massive adhesions, generalized peritonitis and an accumulation of hemorrhagic ascitic fluid (54).

The most prominent biochemical effect of stable cerium,

when injected into rats, was noted to be liver toxicity in the form of focal hepatic necrosis and fatty liver degeneration, with the female being more susceptible than the male (8,57,58,59,60,61,62).

Cerium did not induce fatty liver at all in guinea pigs, chickens or dogs; only occasionally in rabbits, and always in rats, mice and hamsters (58). Within 48 hours after an intravenous administration of 2-3 mg./kg. of cerium to rats, the liver became blanched and distended and showed diffuse lesions in the form of fatty infiltration (8,60,61). This fatty degeneration was characterized by a marked increase in neutral esterified fatty acids, but with only little change in the phospholipid or cholesterol level in the liver (58,60,63). Glenn, Tischer and Stein (64) reported that cerium reduced the ability of the affected liver to oxidize fatty acids and that serious mitochondrial injury to liver cells was indicated by a manifest inhibition of oxidative phosphorylation and marked increase in ATP-ase activity. The lowered capacity of fatty livers for oxidation of fatty acids has also been shown by Snyder et al. (65) who reported practically complete inhibition of octanoic acid oxidation by rat liver mitochondria following administration of stable cerium. Further evidence of hepatic damage by cerium was an observed decrease in serum glucose levels (8,59), and a depression of oxygen consumption by liver slices with a concurrent rise in serum alpha and gamma globulins and decrease in serum albumins (61). The fatty liver response to cerium appears to be under hormonal influence. Livers of female

rats were shown to be much more susceptible to cerium toxicity than those of males (58,59). Castration of males resulted in an increased sensitivity to cerium-induced fatty liver, while testosterone administration reduced the fatty infiltration in female rats (8,58). Hypophysectomy decreased fatty liver response in both males and females, while adrenalectomy was effective only in males (8,60,61).

A detailed investigation by Magnusson (8) into the liver toxicity of stable cerium in rats confirmed some former observations and introduced some newer concepts. Hypoglycemia resulting from intravenous administration of cerium was shown to be a result of acute liver damage. Likewise, a sharp increase in the blood serum concentration of ornithine-carbamyl-transferase (OCT) following cerium injection was used as an indication of serious hepatic injury. Focal necroses were observed, particularly in the caudal lobe where blood supply was possibly inadequate due to hepatic edema. Electron microscopic visualization of fatty liver cells indicated that the cytoplasmic matrix was compressed by swollen mitochondria, lipid droplets and large vacuoles. The nuclei showed degenerative changes and lost their normal round shape. Injury to the mitochondria of liver cells was considered to elicit the fatty hepatic degeneration resulting from cerium administration (8).

An <u>in vitro</u> study, comparing the effects on selected enzymes involved in the fatty liver response, showed that such enzymes were inhibited to varying degrees (66). This action

of cerium appeared to be similar to that elicited by other heavy metals.

High predominance among nuclear fission products has attracted urgent attention to the radiotoxicity of cerium isotopes (3,5,8,10). Of some 13 cerium radionuclides formed during uranium-235 fission, cerium-144 is the most hazardous from the biological viewpoint. Besides its high yield (approximately 5 per cent) in fission, cerium-144 has the longest decay half-life (290 days) of all cerium isotopes formed (3,4). Cerium-144 decays by emission of gamma rays and beta particles (0.32 Mev) to a radioactive daughter, praseodymium-144, which in turn emits powerful beta particles with maximum energies of 2.29 and 2.98 Mev (17). Thus internal deposition of cerium-144 can result in tissue exposure to high energy beta particles.

Reports of the radiotoxicity of cerium isotopes have indicated that occurrence of tissue damage from radioactivity preceded any adverse effects due to the presence of stable elemental cerium (63,67,68). As may be expected, cerium radiotoxicity was manifest in radiation damage to organs concentrating the isotope. Topical application of cerium-144 to rats was shown to produce skin tumors (67) and oral administration resulted in significant changes in gastric and intestinal vascular systems with subsequent tumors, particularly in the large intestine (69). Soluble cerium-144 as $CeCl_3$ solution was found to be a pulmonary carcinogen when intratracheally injected into rats in doses as low as 10 μc per rat (67). Inhalation of cerium-144 oxide by dogs resulted in

intense lethal radiation damage to the lungs (70). Post-mortem examination of the animals also revealed radiation injury to hepatic and bone tissue, illustrating the absorption of radiocerium via the pulmonary route. After parenteral administration of cerium-144 to rats, cirrhosis of the liver was detectable within 50 days and neoplasms originating in the epithelium of the intrahepatic bile ducts developed some 100 to 200 days later (71).

Skeletal deposition of cerium-144 has been shown to result in an exposure of bone tissue to an effective equivalent of over 6 Mev (72). Thus, concentrations of radiocerium in vascular-rich, actively growing areas of bone manifest radiotoxicity in the form of bone carcinogenesis and depressed hematopoiesis (47,73). McFee (74) administered cerium-144 to female rats from day 4 to 14 of gestation. High embryonic absorption resulted in markedly reduced numbers of young, with physical defects occurring in the eyes and testes of the surviving progeny.

8. Specific Physiological Effects of Cerium

Cerium has been shown to produce a high degree of ophthalmic irritation. When applied to rabbits' eyes, a solution of stable $CeCl_3$ produced conjunctival ulcers and opacification of the denuded cornea (63,75). Pulmonary irritation was demonstrated by the appearance of transient chemical pneumonitis, subacute bronchitis and focal emphysema after intratracheal injection of rare earth oxides and fluorides in guinea pigs (76). No injury or irritation to the intact skin was produced by cerium; however, application

to abraded skin resulted in epilation and scar formation (63). Of greater importance, as far as skin lesions were concerned, was the production of granulomas from intradermal injections of stable rare earth chlorides (77). Gabbiani, Jaqmin and Selye (78) have reported the production of soft tissue calcifications by cerium. Following intravenous administration of stable $CeCl_3$, splenic calcification was noted, while subcutaneous injection of the rare earth induced connective tissue calcification at the subcutaneous site. Gabbiani *et al.* (78) further observed that intravenous administration of cerium or any other rare earth, produced topical thrombohemorrhagic lesions at sites of epinephrine injection. Apparently, after treatment with the rare earth metals, the catecholamine could create local conditions which greatly favored topical thrombus formation even when the clotting of circulating blood had been inhibited by effective anticoagulants.

9. Alteration of Bone Mineral Metabolism by Dietary Factors

The various skeletal deformations and alterations in bone metabolism produced by a rachitogenic diet or changes in dietary calcium-phosphorous levels have generally been accepted, and have recently been reviewed in a series of volumes edited by Comar and Bronner (79). The effects of vitamin A-deficiency upon bone formation have also been recognized and have been presented in several volumes (80,81).

Since the discovery that some radionuclides formed during the process of nuclear fission are definite bone-seekers with prolonged skeletal retention, numerous methods of hastening radiometal mobilization from these sites have been attempted.

Because varying dietary intakes of calcium, phosphorous and vitamins A and D are able to produce profound alterations in bone metabolism, such deficiency diets have been used to study mobilization and deposition of radiometals in the skeleton. Numerous reports in the literature have recently dealt with attempts to mobilize radiostrontium, a pronounced bone-seeker, by means of dietary factors (82,83,84,85). The skeletal deposition of both strontium-90 and calcium-45 was found to be inversely proportional to the calcium content of the diet (86,87,88). Harrison and Frazer (89) reported that a diet deficient only in calcium increased the accumulation of both administered strontium and calcium in the skeleton of growing rats. Feeding a high-calcium diet to the animals after administration of radiostrontium was shown to be effective in reducing the skeletal burden of the isotope, although the degree to which the radiostrontium was mobilized from the skeleton varied somewhat with each report (84,87,90, 91).

Harrison (92) reported that a phosphorous-deficient diet for 20 days prior to injection of calcium-45 and strontium-89 in rats reduced the skeletal retention of each isotope. Furthermore, an increase in the dietary phosphorous level after administration of the radioactive calcium and strontium resulted in lowered excretion and prolonged skeletal retention of these isotopes. Kriegel et al. (93) also noted that a reduction in the radiostrontium content of bone was induced by a calcium-rich, phosphorous-deficient diet, fed either before or after injection of the radioisotope.

Van Dutten (82) showed that a low-phosphorous diet, following skeletal deposition of strontium-90, was effective in decreasing the skeletal retention of the radiostrontium in mice.

Alterations of the dietary calcium-phosphorous ratio together with a lack of vitamin D, were employed to produce animal rickets for study of radioactive mineral incorporation into bone (94). Jones and Copp (95) noted an initially rapid uptake of strontium-89 in rachitic rats, followed by a rapid release of the isotope from the animals. Other studies with rachitic animals illustrated a decreased calcium-47 and strontium-90 uptake in bone with a concurrent rise in urinary excretion of these isotopes (94,96).

Several workers have reported on the absorption and retention of bone-seeking isotopes such as radioactive calcium and strontium, as a function of the age of the experimental animal. Studies on dogs (97,98) as well as on mice and rats (95,99,100) have all indicated that young immature animals with actively growing skeletons retain radiostrontium and calcium to a much greater degree than do mature animals. Furthermore, modifications in dietary levels of calcium and phosphorous were noted to affect the deposition and mobilization of radioactive strontium and calcium isotopes much more readily in young animals than in mature animals (83,100).

Alterations of calcium and phosphorous levels in the diets of rats were also shown to affect the rate of absorption, tissue distribution, and the turnover of zinc-65 (101). Dietary amounts of calcium and phosphorous, as well as their ratios, have been demonstrated to have an influence on

magnesium metabolism (102). A high phosphorous intake reduced the absorption of magnesium even when the diet was ample in this element (103). The bone ash of phosphorous-deficient rats showed a 2-1/2 fold increase in concentration of an administered fluoride dosage over that of normal animals (104).

Massive doses of vitamin A accelerated the process of bone resorption and mobilized such bone-seeking radionuclides as strontium-85 and lead-210 (105). However, an excess of vitamin A caused an increase in the skeletal deposition of administered cerium-141 in rats (106).

10. EDTA in Radiometal Mobilization

The discovery of the complexing properties of ethylenediamine tetra-acetic acid (EDTA) introduced a great interest in the removal of foreign radioactive elements from the body by means of various chelating agents. Numerous reports appeared in the literature with favorable prospects for use of EDTA in accelerating the excretion of radioelements, especially the isotopes of yttrium and plutonium (107,108, 109). Catsch et al. (110,111,112) have shown that EDTA was effective in lowering the levels of radiocerium in the liver, kidneys and spleen, and that it was not effective in reducing the skeletal concentrations of the cerium. On the contrary, EDTA was found to produce a slight increase in the retention of radiocerium in bones. A comprehensive review on the removal of potentially hazardous radionuclides from the mammalian body by EDTA and numerous newly developed chelating agents has recently been presented by Catsch (113).

EXPERIMENTAL METHODS AND MATERIALS

1. Chemical Analysis of Cerium

Early in this investigation, an attempt was made to adapt or refine a method of chemical analysis which would be sufficiently sensitive to determine the concentration of elemental cerium in various tissues following administration. Because an intravenous dose as low as 2.5 mg./kg. was close to the $L.D._{50}$ of cerium in rats, a method with a sensitivity in the sub-microgram range was desirable. The analytical chemistry of the rare earths has been reviewed by Kyker (20) and Vickery (114). Of a number of analytical methods investigated in the present study, the most sensitive appeared to be the spectrophotometric determinations of cerium employing Arsenazo III (115), and a potassium carbonate complex method (116). However, in each of these methods, as well as in any others, the presence of other elements interfered with the accurate determinations of cerium levels in biological samples. Thus it was decided to utilize a radioactive method of analysis.

2. Animal Procedure

Throughout this investigation, all animals used were male albino rats of the Sprague-Dawley strain. Weanling rats weighing between 50 and 55 gm. were divided into groups of test and control animals and housed under the same conditions. The control animals were maintained on Purina Laboratory Chow and tap water ad libitum. The test animals were sustained on

the specified deficiency diets and demineralized water. In the study involving the rachitogenic diet, both control and test animals were kept in a darkened room throughout the experiment.

In the time study, animals were sacrificed at intervals of 1, 3, 5 and 21 days, whereas in the investigation of dietary effects, a time interval of 5 days after injection was selected.

3. Test Diets

The test diets were obtained from General Biochemicals, Chagrin Falls, Ohio, and were stated to have the following compositions:

Vitamin A-Deficient Test Diet

Prepared according to the specifications of U.S.P. XIV.

Ingredients	Composition
Vitamin-Free Test Casein	18.0%
Starch	65.0
Cottonseed Oil	5.0
Salt Mix, U.S.P. XIV	4.0
Yeast, Dried, U.S.P.	8.0
Vitamin Supplements	**gm./100 lbs.**
Vitamin D (Viosterol)	0.5

Rachitogenic Test Diet U.S.P.

Prepared according to the specifications of U.S.P. XIV.

Ingredients	Composition
Ground Yellow Corn	76.0%
Gluten, Ground	20.0

Rachitogenic Test Diet (continued)

Ingredients	Composition
Calcium Carbonate	3.0%
Sodium Chloride	1.0

Ca/P = 5.85

Calcium-Deficient Test Diet

Is compounded with a special salt mix resulting in minimum calcium content.

Ingredients	Composition
Whole Dried Egg (ether extracted)	27.0%
Sucrose	10.0
Lard	10.0
Dried Yeast	7.0
Cod Liver Oil	2.0
Starch	40.0
Salt Mix	4.0

Salt Mix	gm./100 lbs.
Ferric Citrate	29.462
Magnesium Chloride	855.122
Manganous Sulfate	.414
Potassium Alum	.190
Potassium Chloride	355.491
Potassium Citrate	371.976
Potassium Iodide	.085
Potassium Sulfate	37.620
Sodium Chloride	164.430
Sodium Fluoride	1.048

Low-Phosphorous Test Diet

Ingredients	Composition
Purified Beef Blood Fibrin	20.0%
Cane Sugar	60.0
Vegetable Oil (hydrogenated)	10.0
Cod Liver Oil	2.0
Salt Mix and Vitamin Supplements	8.0

Salt Mix	gm./100 lbs.
Sodium Chloride	454.0
Potassium Chloride	682.0
Magnesium Sulfate	136.2
Ferric Citrate	45.4
Calcium Carbonate	454.0
Copper Sulfate	5.902
Manganous Sulfate Monohydrate	2.034
Zinc Carbonate	.7050
Cobalt Chloride	.4080
Potassium Iodide	.3640
Cane Sugar	444.5560

Vitamin Supplements	gm./100 lbs.
Thiamine HCl	.2540
Riboflavin	.2540
Pyridoxine HCl	.2770
p-Aminobenzoic Acid	.2540
Nicotinic Acid	2.2700
Calcium Pantothenate	2.2700
i-Inositol	9.0800
Choline Chloride	45.4000
Cane Sugar	393.9100

4. Preparation of Radioactive Cerium Solution

Cerium-141 was obtained from Atomic Energy of Canada Limited in the form of CeO_2 powder. The irradiated target unit containing 100 mg. CeO_2 had an activity of approximately 10 millicuries. The oxide was dissolved in a mixture of 3 ml. concentrated nitric acid and 2 ml. of a 30 per cent hydrogen peroxide solution with the aid of gentle heat, then was further heated to decompose the remaining peroxide. A stock solution was prepared by diluting the cerium-141 nitrate to 20 ml. with distilled water. The solution for injection was prepared by adding 100 mg. of citric acid to 5 ml. of the stock solution of cerium-141 nitrate, adjusting to pH 7.0 with sodium hydroxide solution, diluting to 20 ml. with distilled water, and transferring to a rubber cap multidose vial. This solution was kept refrigerated when not in use.

5. Experimental Procedure

The cerium-141 citrate complex was administered intravenously via the tail vein. The dosage used was 15 μc / kg. and the volume injected never exceeded 0.5 ml. per rat. At the time of injection, using the same syringe, an exact volume of the same cerium-141 solution was quantitatively transferred to a counting vial and made up to 3 ml. with water. Several such samples were measured and set aside to be used as reference standards indicating the amount of radioactive cerium that was injected into each animal. Correction for radioactive decay was effected by counting the prepared standards concurrently with the tissue samples.

The animals were housed in stainless steel metabolism cages with free access to the specified diet and water. The

urine and feces were collected separately throughout the duration of each experiment. The loose, tar-like consistency of the feces excreted by the rats on a phosphorous-low diet hindered their quantitative removal from the metabolism cages. Consequently, the bottoms of the cages were lined with filter paper and this paper, together with the adhering fecal material, was wet ashed for sampling.

At specific intervals after injection, the animals were sacrificed by decapitation, and a blood sample collected into a beaker containing a small amount of heparin. The tissuesto be investigated were excised, blotted free of blood, cleared of any adhering adipose or extraneous material and placed in tared beakers for immediate weighing. In the preliminary study of cerium-141 uptake by soft tissues, the animals were sacrificed 12 hours and 24 hours after injection and the following organs removed: entire liver, spleen, brain, heart, prostate, seminal vesicles, both adrenals, thyroid glands and one of each of the lungs, kidneys and testicles. In all subsequent experiments, only liver, spleen, kidney and bone samples were considered and were obtained as mentioned above.

6. Tissues Examined for Cerium-141 Uptake

The following organs were examined for their uptake of radioactive cerium:

a. The whole spleen.

b. The entire right kidney.

c. Two portions of about 500 mg. from the left lateral lobe of the liver which had been accurately weighed. Each portion was quantitatively weighed and analyzed separately for purposes of duplication.

d. The lower end of each femur which was scraped free of muscle and connective tissue. These sections of bone were considered to represent the uptake of cerium-141 in the epiphyseal portion of the skeleton. The bone section from each femur was analyzed separately for purposes of duplication.

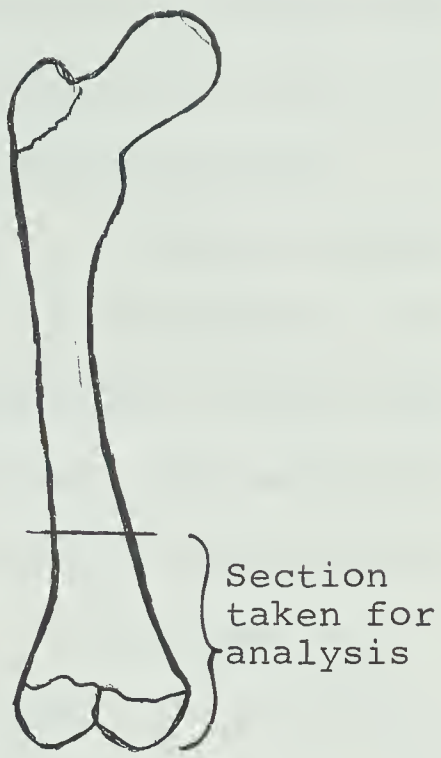

Figure 1
Femur of Rat

e. The tibia-fibula complex from both hind limbs after removal of the epiphyseal ends. This was considered to illustrate the deposition of cerium-141 in the bone shaft, or diaphysis of long bone.

Figure 2
Tibia-fibula complex
of rat

7. Preparation of Tissues for Counting

The tissues examined were wet ashed in concentrated nitric acid with the aid of gentle heat and made up to a 10 ml. volume with the acid. A 3 ml. aliquot of each sample was transferred to a counting tube for radioactive analysis.

In the estimation of radiocerium excretion via the urine, the insides of the metabolism cages were sprayed with 10 ml. of hot water and the washings were combined with the urine samples and made up to a volume of 100 ml. A 3 ml. aliquot was then measured into a counting tube. The contents of the gastrointestinal tract were removed by squeezing out the contents of intestinal sections on a glass plate. The material from the gastrointestinal tract together with the cumulative fecal excretion was then collected into a large beaker and 150 ml. of concentrated nitric acid was added. To minimize frothing, the fecal material was set aside for about 20 hours before complete wet ashing by application of heat. After dilution to 250 ml., a 3 ml. aliquot was withdrawn for counting.

Since radiocerium was found to be readily adsorbed onto glass surfaces, all glassware used in this investigation was previously siliconized according to the procedure given by Chase and Rabinowitz (117).

8. Radioactive Analysis

Cerium-141 decay into stable praseodymium proceeds by the emission of beta particles with maximum energies of 0.44 and 0.58 Mev accompanied by gamma rays with an energy of 0.145 Mev (17). The detection of gamma rays by means of a thallium-activated NaI crystal scintillation system affords a reliable measurement of the level of cerium-141 in a specimen.

A RIDL model 24-20 400 channel pulse height analyzer[a]

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[a]Nuclear Chicago Corporation, Chicago, Illinois, U.S.A.

was used to confirm the radiochemical purity of each shipment of cerium-141 dioxide. The 3 ml. aliquots of tissue samples along with the previously prepared standards were each counted for 3 minutes in a Nuclear Chicago Model 4219 Automatic Well Scintillation Detector[a]. The counts obtained were corrected for background radiation. The various samples were counted in the integral mode with the lower discrimination adjusted to reject energies below 60 Kev.

9. Autoradiography

Autoradiographs of the rat femurs were prepared in an attempt to localize the sites of radiocerium deposition in bone. A dosage of approximately 50 μc of cerium-141 as a citrate complex was administered intravenously via the tail vein into several young rats. The animals were sacrificed after 1, 3 and 15 days, and the entire femurs removed and scraped free of all muscle and connective fibre.

An International Equipment Model CT Microtome Cryostat[b] was used for freezing and sectioning of the bone. The entire femur was affixed to the specimen plate holder by copious applications of O.C.T. carbowax[c], and subsequent quick freezing in the cryostat. It was found that the separation of the bone shaft from the marrow during sectioning could be prevented by embedding the bone completely in the carbowax. Longitudinal sections of femur, 32 microns thick were obtained.

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[a]Nuclear Chicago Corporation, Chicago, Illinois, U.S.A.
[b]International Equipment Company, Limited, Needham, Mass., U.S.A.
[c]Lab-Tek, Westmount, Illinois, U.S.A.

In the darkroom, the bone sections were placed on Kodak NTB-3 Nuclear Track Plates[a] (25 microns thickness, 1 micron gelatin top) and covered with siliconized glass slides. The glass slides and the photographic plates were then clamped together, using an Esterbrook No. 2 clamp, placed in light-tight cassettes containing a drying agent[b] and stored in a freezer at about -8°C. for 14 days.

All solutions used in developing the plates were maintained at a temperature range of 19° to 21°C. The plates were washed in distilled water for 30 seconds to remove excess carbowax, immersed in a Kodak D-19 developer solution for 10 minutes, rinsed again in distilled water for 30 seconds and then transferred to the Kodak acid fixer for 20 minutes after which the plates were washed in water for a half hour and finally air-dried. The intense artifacts which were produced by the O.C.T. carbowax were found to be eliminated by dipping the plates in distilled water at 20°C. several times prior to immersion in the developing solution.

Photomicrographs of the autoradiographs were obtained by means of an American Optical Series 10 phase microscope and a Polaroid MP-3 Land Camera using PolaPan 200/Type 42 film.

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[a]Kodak Specialized Products Division, Eastman Kodak Company, Rochester, N. Y., U.S.A.

[b]Drierite, W. A. Hammond Company, Xenia, Ohio, U.S.A.

10. Statistical Procedures

In the time uptake study and the investigation into the various parameters affecting the distribution and excretion of radiocerium, the mean, standard deviation of the mean, and standard error of the mean were calculated by standard statistical procedures. Differences between control and test animals were calculated and compared by Student's "t" test.

RESULTS AND DISCUSSION

1. Preliminary Investigation into the Uptake of Cerium-141 by Various Soft Tissues of the Rat

Shortly after an intravenous administration of radiocerium, the most notable feature observed was the rapid decline in blood concentration. Within one hour after injection, the blood level was only 1 per cent of the original administered dose per ml. of plasma, and after 12 hours, the levels further dropped to 0.05 per cent per ml. of plasma and to 0.03 per cent per ml. of whole blood. This abrupt clearance of blood levels was paralleled by an intense uptake by the liver. Thus, within 1 hour, about 90 per cent of the injected amount was concentrated in this organ with only a slight decline in the first 12 hours. One hour after intravenous administration, the spleen and kidneys contained about 5 and 3 per cent of the dosage, respectively, and diminished to 3 and 2 per cent after 12 hours.

In this preliminary investigation of cerium-141 uptake by soft tissue in the rat, careful excision and analysis for radioactivity in the entire lung, heart, brain, prostate, seminal vesicles, testes and thyroids indicated that none of these organs concentrated the radiocerium to any appreciable extent. Twenty-four hours after intravenous administration, the level of radioactivity in each of these organs was below 0.1 per cent of the injected dose and subsequently decreased to lower levels. The radiocerium content of skeletal muscle appeared to coincide with the levels of cerium-141 in the blood supply of the muscle. The adrenal glands, although weighing approximately only 30 mg., displayed a strong uptake

per unit weight, thus accounting for 0.18 per cent of the total activity after 12 hours. This initial high concentration was found in a preliminary experiment to be contained mainly in the adrenal cortex. However, after 24 hours, the level of cerium-141 in the whole adrenals declined rapidly. Thus, after the first day, the soft tissues of importance in the uptake of radiocerium were the liver, spleen and kidney.

Because of the intense uptake of radiocerium by the entire liver, only several tissue slices were taken for analysis. It was then necessary to determine whether the cerium was evenly distributed throughout the whole organ. In rats, a gall bladder is absent and tributary ducts from the various lobes unite to form the bile duct (118). A generous portion of liver containing the common bile duct and the larger tributaries from each lobe was excised and the remainder of the median, right lateral, left lateral and caudate lobes were divided into portions of about 500 mg. and analyzed separately for cerium-141 content. Distribution of radiocerium appeared to be uniform throughout all of the four lobes of the liver. However, any contamination of the liver portion with excess bile resulted in a greatly increased radioactivity in that sample.

2. Time Study of the Distribution and Excretion of Cerium-141

A time study of the metabolism of cerium-141 after intravenous injection indicated a definite pattern of distribution: a high uptake by the liver and skeleton, and excretion mainly via the feces (Tables 1,2,3,4; Figs. 3,4,5,6). One day after injection, about 86 per cent of the administered dose of

TABLE 1

Distribution and Excretion of Cerium-141 One Day After Intravenous Injection[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean[d]	86.45	3.08	0.54	1.64	1.22	0.44	4.98
S.D.[e]	6.42	0.56	0.54	0.45	0.23	0.11	1.45
S.E.M.[f]	2.87	0.25	0.07	0.20	0.10	0.05	0.65

a. Cerium-141 administered as a citrate complex, 15 μc/kg.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Data from 5 animals. For individual values, see Appendix (Table I).
e. Standard deviation of the mean.
f. Standard error of the mean.

TABLE 2

Distribution and Excretion of Cerium-141 Three Days After Intravenous Injection[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean[d]	70.82	1.80	0.63	1.99	1.36	0.54	11.89
S.D.[e]	9.95	0.85	0.23	0.87	0.76	0.21	5.26
S.E.M.[f]	4.06	0.39	0.09	0.36	0.31	0.09	2.15

a. Cerium-141 administered as a citrate complex, 15 μc/kg.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Data from 6 animals. For individual values, see Appendix (Table II).
e. Standard deviation of the mean.
f. Standard error of the mean.

TABLE 3

Distribution and Excretion of Cerium-141 Five Days After Intravenous Injection[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean[d]	67.38	1.95	0.69	2.33	1.39	0.52	14.95
S.D.[e]	6.61	0.83	0.24	0.51	0.45	0.20	3.07
S.E.M.[f]	2.50	0.31	0.10	0.19	0.17	0.08	1.16

a. Cerium-141 administered as a citrate complex, 15 μc/kg.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Data from 7 animals. For individual values, see Appendix (Table III).
e. Standard deviation of the mean.
f. Standard error of the mean.

TABLE 4

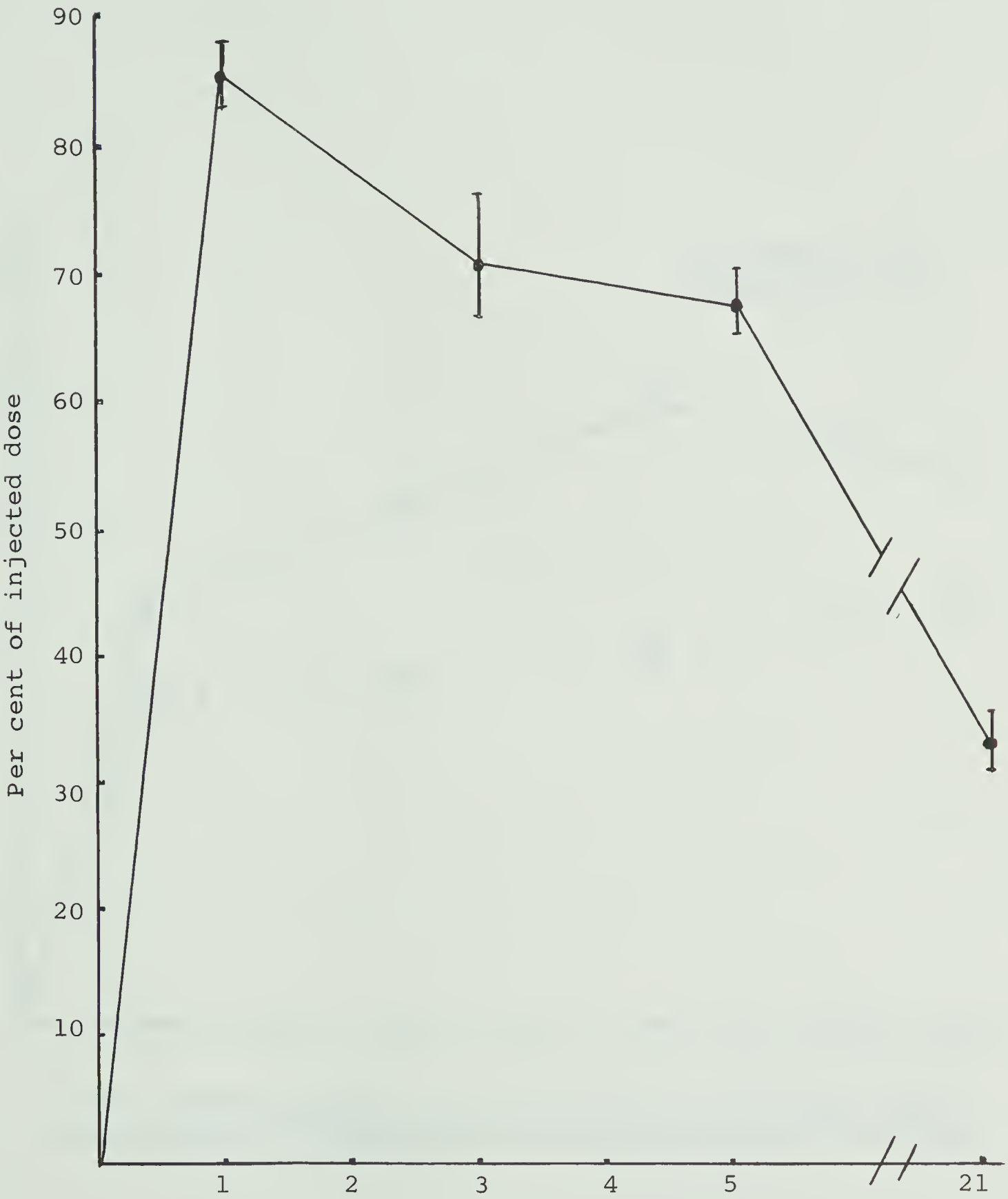
Distribution and Excretion of Cerium-141 Twenty-One Days After Intravenous Injection[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean[d]	32.29	1.35	1.17	2.14	1.67	1.21	41.56
S.D.[e]	3.72	0.59	0.31	0.44	0.12	0.56	2.94
S.E.M.[f]	2.15	0.34	0.18	0.25	0.07	0.32	1.70

a. Cerium-141 administered as a citrate complex, 15 μc/kg.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Data from 3 animals. For individual values, see Appendix (Table IV).
e. Standard deviation of the mean.
f. Standard error of the mean.

Figure 3.

Uptake of Cerium-141 by the Entire Liver

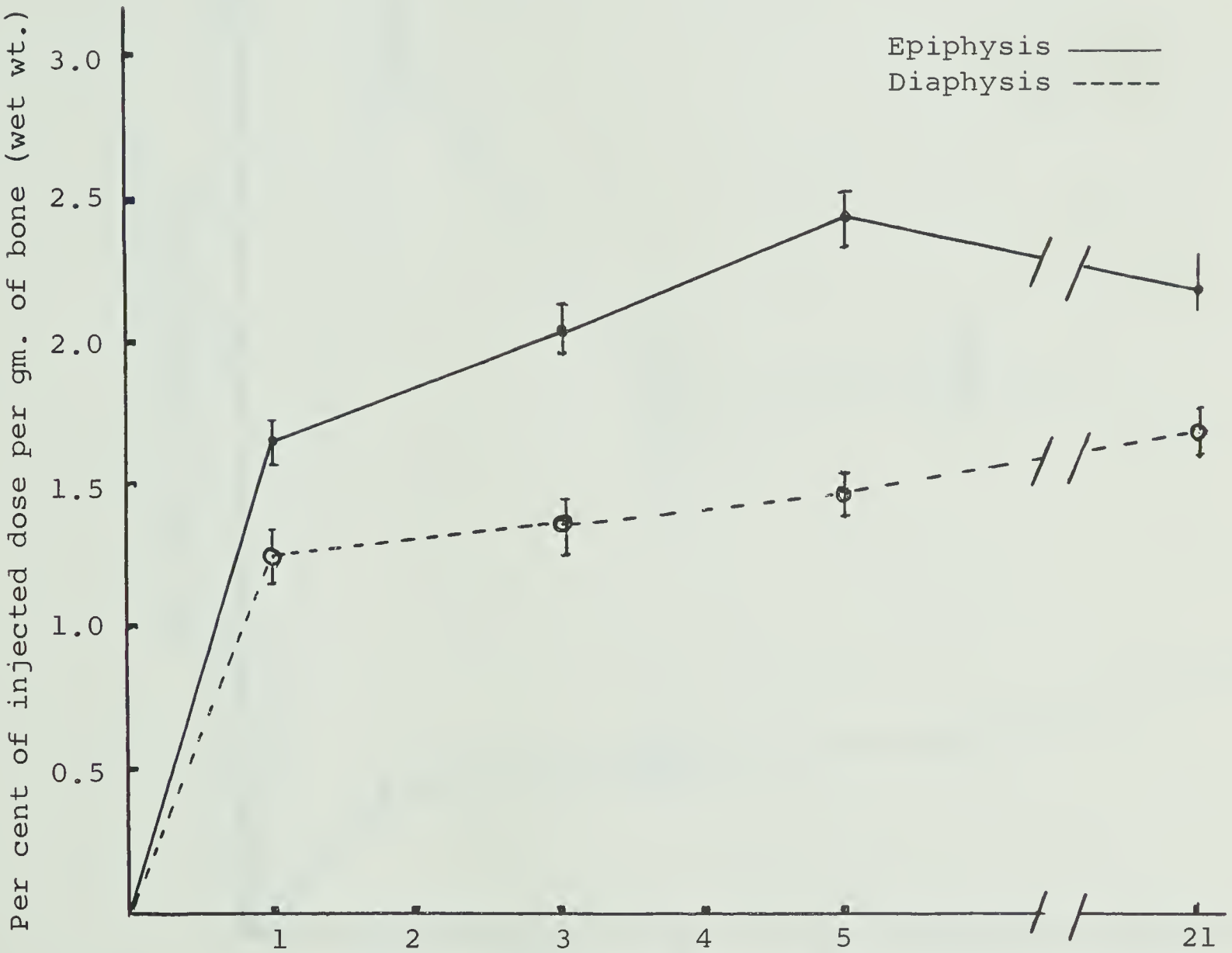


Time intervals after intravenous injection of cerium-141 (days).

Vertical bars ⌶ represent the standard error of the mean.

Figure 4.

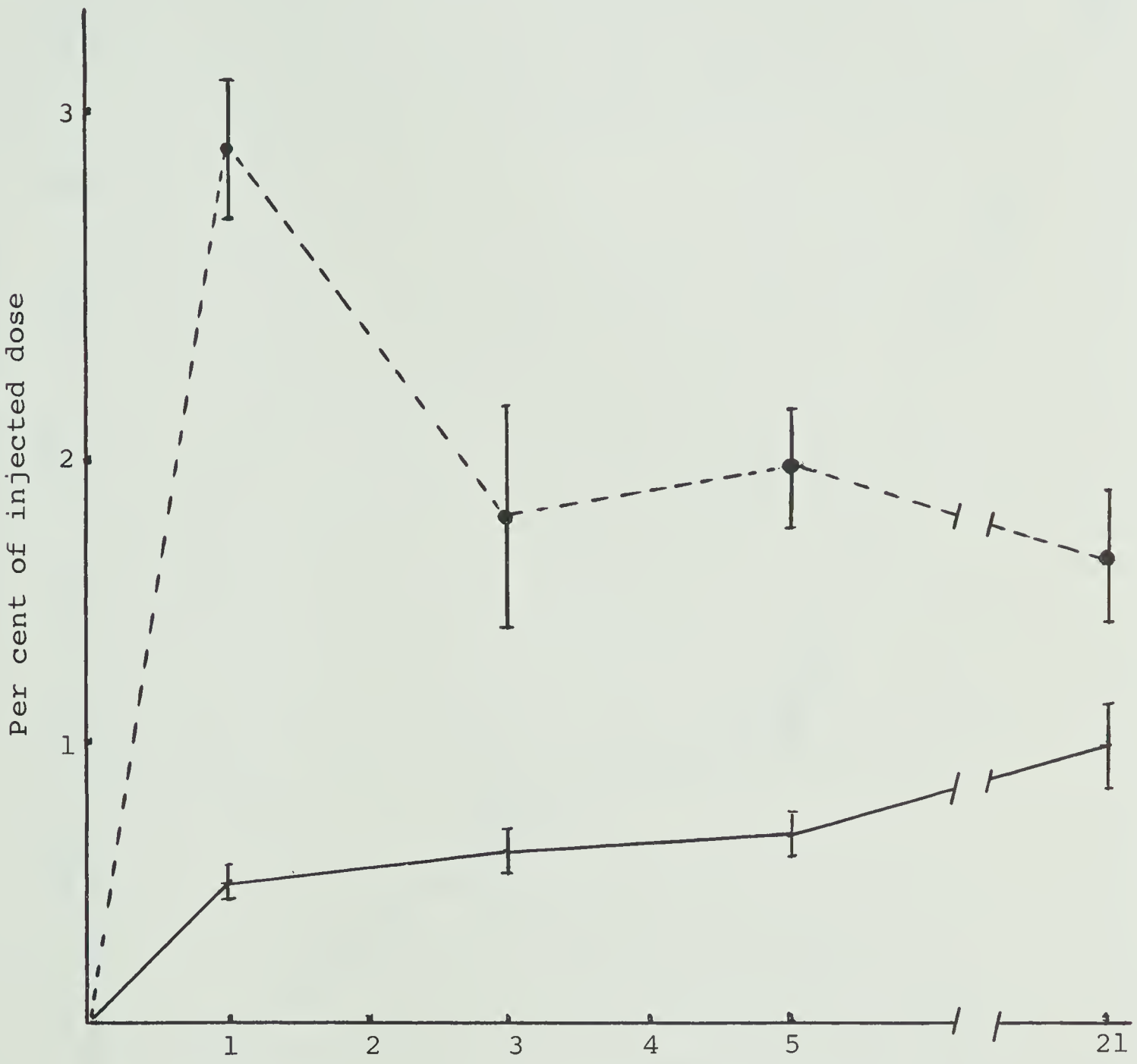
Concentrations of Cerium-141 in Bone



Time intervals after i.v. injection of cerium-141 (days).
Vertical bars I represent the standard error of the mean.

Figure 5.

Uptake of Cerium-141 by the Entire Spleen and Both Kidneys



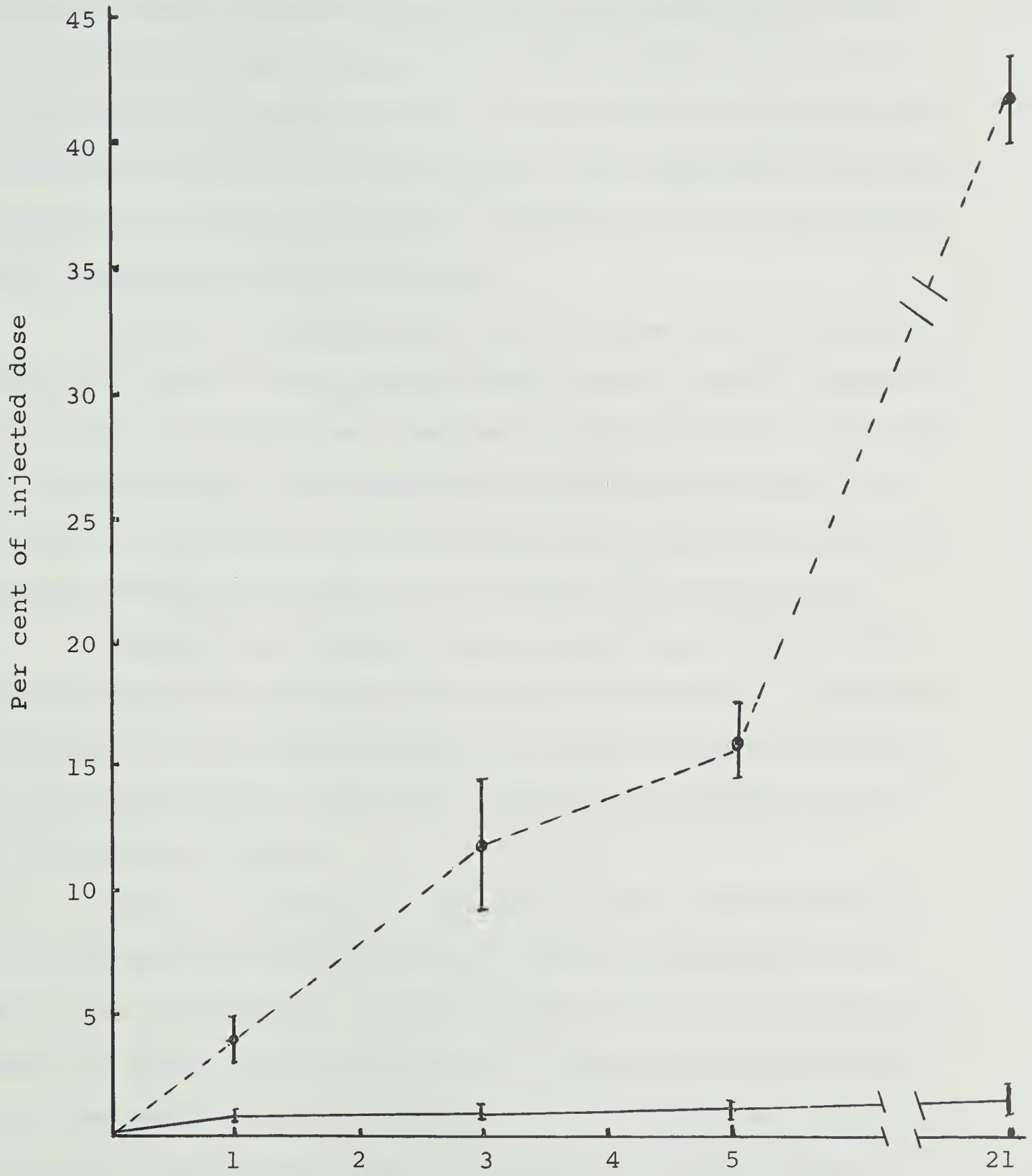
Time intervals after intravenous injection of cerium-141 (days).

Entire Spleen ---- Both Kidneys ———

Vertical bars I represent the standard error of the mean.

Figure 6.

Cumulative Excretion of Cerium-141



Time intervals after intravenous injection of cerium-141 (days).

Urinary Excretion ——— Fecal Excretion and G.I.T. Contents -----

Vertical bars I represent the standard error of the mean.

radiocerium was concentrated in the liver. This level declined gradually to 32 per cent after 21 days, indicating that the biological half-life of cerium-141 in the organ was approximately 15 days. Durbin et al. (7) administered a cerium-144 citrate complex intramuscularly and reported a similar rate of hepatic excretion, but Catsch et al. (112) employing cerium-144 chloride intravenously obtained a somewhat faster elimination with a half-life of about 10 days.

Levels of radioactivity in the spleen initially reached about 5 per cent of the injected dose within 1 hour, dropped to 3 per cent in 12 hours and remained at that level for the first day. After 3 days, the spleen was found to contain only 1.8 per cent of the entire dose, and this level appeared to remain somewhat constant throughout the length of the experiment.

Although the kidneys concentrated 3 per cent of the entire dosage in the first hour after administration, the concentration of the element dropped to only 0.5 per cent after 1 day, and subsequently rose very gradually to slightly above 1 per cent after 21 days.

Skeletal retention of radiocerium was represented by the analysis of two types of bone tissue: the growing end of long bones or epiphysis, and the compact bone of the diaphysis. Because actively growing young rats (weighing about 120 gm.) were selected for this experiment, it was found that cerium-141 was readily incorporated into the osseous tissue. One day after injection, the concentration of radiocerium in the epiphysis was 1.6 per cent of the injected dose per gm. of fresh bone. This concentration rose to 2.3 per cent per gm. in

5 days and decreased only slightly after 21 days. The corresponding amounts of cerium-141 in the bone shafts were somewhat lower than those in the epiphyses. However, a steady increase in the diaphyseal levels was noted throughout the 21-day term of the study. Analysis of several bone samples consisting of the lower jaw and teeth indicated that this portion of the skeleton concentrated radiocerium to a lesser extent than did the long bones.

Urinary excretion of radiocerium was low. It appeared that about 0.5 per cent was excreted via the urine on the first day but the total cumulative excretion after 21 days was only 1.2 per cent of the injected dose. Individual variations in the urinary excretions may possibly have been caused by small amounts of fecal contamination.

Excretion of radiocerium in the rat was mainly via the feces. Analysis of the cumulative fecal excretion together with the contents of the gastrointestinal tract showed that the fecal elimination of cerium-141 was about 3.6 per cent after the first day and rose steadily to over 40 per cent after 21 days.

Fairly wide variations were noted both in levels of cerium-141 in the various tissues and the amount of excreta among the individual animals, particularly 1 and 3 days after injection. However, after 5 days, variations were somewhat less and the radiocerium levels in the skeleton appeared to be well-established. Hence, all further studies involving various parameters affecting the distribution and excretion of cerium-141 were carried out 5 days after injection.

Discussion

The high uptake of radiocerium by the liver may be partially ascribed to radiocolloid formation and subsequent retention by the reticuloendothelial system. In low concentrations, the rare earth metals, including cerium, have been shown to form colloids at physiological pH (8, 119). Upon intravenous injection, the cerium-141 citrate, being a fairly weak chelate, probably dissociated, releasing ionic cerium which could readily combine with hydroxyl, phosphate and carbonate ions to form colloidal aggregates (120). Furthermore, since rare earths are known to bind to plasma protein (7,44,121,122), it is conceivable that complexes of radiocerium and proteins were formed. The phagocytosis of these colloidal aggregates, or macromolecules, by macrophages in the reticuloendothelial system may thus account for large deposits of radiocerium in the Kupffer cells of liver and to a lesser degree in the red pulp of the spleen. In addition, the formation of cerium-induced fatty liver (58,59,60) and the subcellular localization of radiocerium in liver cells (8) present evidence that cerium is able to penetrate into liver cells and participate in their metabolism. Aeberhardt *et al.* (51) suggested that the colloidal cerium was initially fixed by the cells of the reticuloendothelial system and that after a short period it was returned to circulation, resulting in secondary fixation in bone tissue and transfer to the hepatocytes. The rapid decline in splenic concentrations of cerium-141 and the gradual accumulation in bone noted in this investigation may well substantiate Aeberhardt's proposal.

The initially high appearance of radiocerium in the kidneys and subsequent rapid clearance appears to have paralleled the blood content of cerium-141. Autoradiographic evidence has shown intense uptake in the renal cortex within 20 minutes after intravenous injection, followed by a rapid clearance (9). The fact that cerium-141 was only slightly excreted in the urine suggests that the element remained firmly bound to the plasma proteins and did not pass through the glomerular filter. Additionally, most of the urinary radiocerium was excreted shortly after injection, suggesting that some ionic form from the injected cerium-citrate complex might still have been present in the circulation in an unbound form.

The predominance of radiocerium excretion via the feces has been mainly ascribed to elimination from the liver through the bile as indicated by experiments involving catheterization of the bile duct (8,53). However, in the present study, cerium-141 was found in the gastric contents shortly after intravenous administration, thus indicating excretion through the gastric mucosa. Recent autoradiographic evidence has also shown an intense localization of radiocerium in the superficial gastric mucosa as early as 20 minutes after intravenous injection (8). Fecal excretion results expressed in the present investigation include the combined contents of both excreta and gastrointestinal contents. Durbin et al. (7), also administering a cerium citrate complex but analyzing the activity in the feces only, reported excretion levels of 1.0, 6.0, and 38.0 per cent of the injected dose after 1, 5 and 20 days, respectively. In this investigation, corresponding levels of cerium-141 were found to

be 3.6, 14.9 and 41.5 per cent of the original amount after 1, 5 and 21 days. It appears the radiocerium remaining in the gastrointestinal contents would account for the wide differences in the 1 and 5 day cumulative excretion levels.

Concentrations of cerium-141 in the bone samples were noted to increase or remain high during the entire length of observation. Since bone portions from different parts of the skeleton, i.e., epiphyseal, diaphyseal and dental osteoid tissue, appeared to concentrate the isotope in varying amounts, the per cent of injected cerium-141 per unit weight of skeleton was probably a composite of the levels in the various types of bone tissue. Previously published data have established skeletal wet weight in rats weighing between 100 and 160 gm. to be about 7 per cent of body weight (123). Accordingly, the skeleton of a 120 gm. rat would weigh approximately 8.4 gm. Using an average of the concentrations found in the epiphyses and diaphyses to represent skeletal uptake, the calculated amount of radiocerium retained by the skeleton can be shown to be approximately 15 per cent after 5 days. Similarly, after 21 days, skeletal retention of radiocerium may be extrapolated to be about 24 per cent of the injected dose. Similar results were obtained by calculating the difference between the total administered dose and the accumulated dose in the liver, spleen, kidney and excreta. Thus, the balance of radioactivity remaining in the animals was considered to be a measure of skeletal retention. Skeletal levels of 14 per cent and 22 per cent of the injected dose after 5 and 21 days, respectively, were calculated by this method. Various workers, employing this same

method, also reported skeletal retention of radiocerium in amounts ranging from 15 to 25 per cent of the injected dose depending on the route and chemical forms of administration (8,61,124). Considering the long-term involvement in bone tissue [estimated biological half-life of 1500 days (10)], it is evident why the skeleton is regarded as the critical target organ for radiocerium. In an attempt to elucidate the incorporation of cerium-141 into bone, an autoradiographic method was employed.

3. Autoradiographic Localization of Cerium-141 in Bones

The skeletal incorporation of radiocerium is associated with the various processes of bone metabolism. The numerous aspects of bone formation, mineralization, growth and maintenance represent an extremely broad field of study and are dealt with in detail in several recent volumes (78, 125,126,127). However, in order to discuss the deposition of cerium-141 in the rat femur as presented in the autoradiographic study, the processes by which growth occurs in long bones will be briefly reviewed.

a. Histology of Bone

Figure 7 illustrates the structure of a typical long bone. The epiphyseal cartilage plate (f) is a portion of the embryonic cartilage and participates in the longitudinal growth of the long bones. The epiphyseal face of the disc is composed of cartilage matrix in which cartilage cells are embedded. These continue to divide, giving rise to new cells, commonly arranged in rows which are separated from one another by cartilage matrix. Replacement of the cartilage cells by

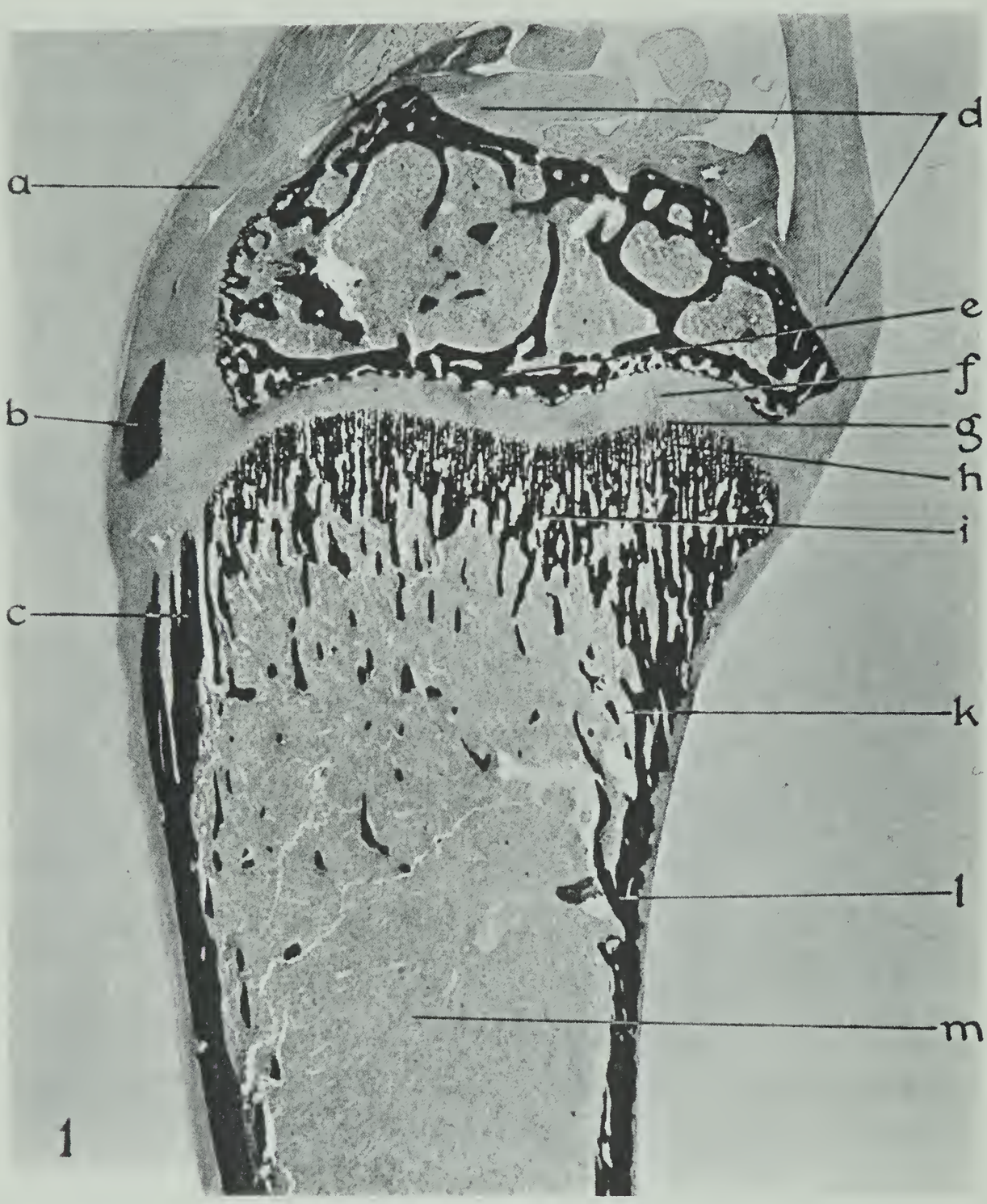


Figure 7

Figure 7. Sagittal section of head of tibia of normal rat. Cut, without decalcification, by method of McLean and Bloom and stained with silver nitrate (von Kossa) to illustrate distribution of calcified tissues. Age 7 weeks, (a) Patellar tendon; (b) ossification center of anterior tibial tubercle; (c) intratendinous ossification in insertion of patellar tendon; (d) insertions of crucial ligaments; (e) area in epiphysis where osteoid is frequently seen; (f) epiphyseal cartilage plate; (g) zone of hypertrophic cartilage and of provisional calcification; (h) primary spongiosa; (i) secondary spongiosa; (k) area at junction of spongiosa with shaft, where osteoid is frequently seen; (l) shaft; (m) bone marrow. Silver nitrate-hematoxylin-eosin.
(Mag. 16X)

Reproduced from "Bone", Figure 1, p. 5 (138).

bone occurs on the diaphyseal side of the disc face, while cartilage grows by apposition on the epiphyseal side. The first indication that the cartilage is to transform into bone is the appearance of hypertrophic alterations within the proliferating cartilage cells (g). When the epiphyseal cartilage and areas directly beneath are viewed in cross section, they resemble a honey comb in structure, each compartment of which contains a column of cartilage cells growing out of the cartilage plate and undergoing hypertrophy. Into each of these columns, there grow, from the bone marrow, minute blood vessels accompanied by perivascular connective tissue. These blood vessels invade the region of the transformed cartilage cells and soon a vascular tissue space (primary center of ossification) is seen (g). In advance of the invading blood vessels, usually to a depth of 2 to 4 cells in the cartilage, the matrix of the walls of the columns starts to calcify. This forms the zone of growth within which the cartilage cells are displaced by the tissue advancing from the bone marrow. The primary spongiosa (h) is formed from a direct continuation of the cartilage matrix. Here blood vessels penetrate the hypertrophic cartilage cells, most of which then disappear and become replaced by vascular growth. Osteoblasts appear which coat the cartilage remnants with layers of bone. In the formation of bone, the osteoblasts become surrounded by bundles of collagenous fibres which are then cemented together to form plates of uncalcified bone matrix or osteoid. Minute crystals of calcium phosphate complexes are deposited in the osteoid. The osteoblasts imprisoned in the matrix are converted to

osteocytes. Thus bone spicules appear as long needle-shaped outgrowths from the surface of the epiphyseal plate. In the epiphysis, many of the spicules persist and become linked and interlocked into trabeculae. The resulting trabecular network forms the spongy bone (i) of the mature epiphysis. In the metaphysis of long bones, the resorption of spicules is extensive so that the secondary spongiosa is subject to continual thinning out and reconstruction. It is here that deposition of new bone on the cores of calcified cartilage matrix is most prominent.

Growth in width of the bone shaft is accomplished by subperiosteal formation of concentric lamellae of bone on the periosteal surface, or outer periphery of the shaft. Simultaneous resorption from the endosteal surface increases the size of the marrow cavity and serves to maintain proportional thickness of the shaft. Osteoblasts appear on the periosteal surface, actively laying down new bone. Osteoclasts associated with bone vessels erode the endosteal surface and thus enter the bony tissue from the marrow cavity, forming hollow channels. These channels later become coated with osteoblasts which deposit matrix after which mineralization proceeds. In this manner, haversian systems are formed.

b. Autoradiography

Longitudinal autoradiographs of rat femurs following skeletal incorporation of cerium-141 (Figs. 8,9,10) illustrated heavy depositions of the isotope beneath the epiphyseal cartilage plate, in the trabecular network of the spongiosa in the epiphysis, on periosteal and endosteal surfaces, and a spotted

Figure 8

Autoradiograph of a longitudinal section (32 microns thick) from the femur of a young rat injected with 50 μc of cerium-141 and sacrificed one day later. A heavy deposition of the isotope is noted in the epiphyses and a lighter accumulation in the endosteal surface of the diaphysis. The radiocerium did not seem to be incorporated into the epiphyseal cartilage. (Mag. 2X)

Figure 9

Autoradiograph of a longitudinal section from the femur of a young rat injected with 50 µc of cerium-141 and sacrificed 3 days later. Depositions of radiocerium are visible on the trabeculae of the spongy bone in the epiphyses. In addition to the accumulation of radioactivity on the endosteal and periosteal surfaces, a characteristic spotty appearance is also noted within the bone shaft. (Mag. 2X)

Figure 10

Autoradiograph of a longitudinal section from the femoral diaphysis of a young rat injected with 50 μc of cerium-141 and sacrificed 15 days later. Scattered points of intense radioactive localization are seen throughout the bone shaft. (Mag. 5X)

uneven distribution throughout the compact bone of the shaft. The epiphyseal cartilage itself (Figs. 8,9,13) did not appear to appreciably concentrate the radiocerium. The uptake of radioactivity by the bone marrow seemed less intense than the deposition on bony surfaces, and the activity indicated within the marrow may probably have been associated with uptake by the reticuloendothelial cells (122).

Shortly after administration, cerium-141 appeared in bone mainly in the epiphyseal ends, as deposits on the trabeculae of cancellous tissue (Fig. 8). At this time, some deposition was visible on the endosteal surfaces but not in the bone shaft. However, in the autoradiographs of bone slices obtained 3 days after cerium-141 injection (Fig. 9), besides the heavy uptake of the isotope by the spongy bone, scattered points of radioactivity were also visible throughout the bone shaft. The autoradiograph of the diaphysis of the femur, 15 days after radiocerium administration (Fig. 10) indicated an intense spotty uptake in cortical bone. The series of autoradiographs (Figs. 8,9,10) substantiate the observed gradual increase of the cerium-141 concentration in the bone shaft with time, as illustrated in Figure 4. The scattered points of radioactivity as seen within the bone shaft (Figs. 9,10) have been noted by a number of workers investigating fission products, and this peculiar spotty distribution throughout the dense cortex of bone has been observed to be characteristic of the rare earth isotopes (6,7,128,129). Microscopic examination (Fig. 11) reveals that these foci of high radioactivity appear in the vicinity of blood vessels. Intense perivascular

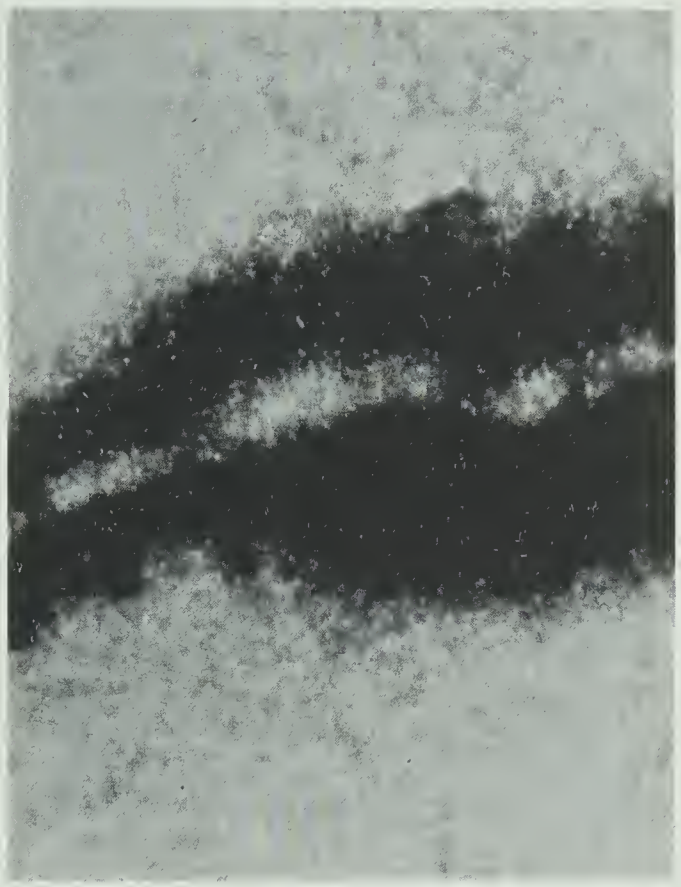


Figure 11

Autoradiograph indicating radioactive deposition along a longitudinal section of a blood vessel, probably a Volkmann's Canal. (Mag. 100X)

localization of cerium-141 is also illustrated in Fig. 12.

The mechanisms by which cerium as well as other rare earth nuclides are incorporated into bone tissue have long been sought. The work of Hamilton (6) in 1947 was one of the earliest reports on the sites of radiocerium localization in bone. Employing autoradiographic methods, Hamilton noted a heavy concentration of radioactivity beneath the periosteum, endosteum, and epiphyseal plate of long bones and interpreted these depositions as an affinity of the radiocerium for the uncalcified osteoid matrix of bone protein. Copp et al. (128,129) and Durbin et al. (7) demonstrated similar distribution patterns in bone with radioactive cerium and other rare earths. These investigators suggested that the lanthanons had a specific affinity for bone protein even in the absence of bone salts and that these elements were actively laid down in the uncalcified organic matrix of bone. Autoradiographs prepared in the present study (Figs. 9,13) also indicate a deposition of cerium-141 in the areas directly above and below the epiphyseal cartilage plate where large quantities of uncalcified osteoid tissue exists. That radiocerium has a definite attraction for bone matrix was also noted in a recent volume on bone seeking elements (130).

However, Jowsey et al. (50) announced that incorporation of cerium isotopes into bone occurs by yet other processes. These workers concluded that radiocerium was deposited in the vicinity of vascular-rich areas on non-growing highly mineralized bone surfaces that were either resorbing or inactive. Such deposition was thought to occur either by surface adsorption or even by an ion exchange process where the rare earth

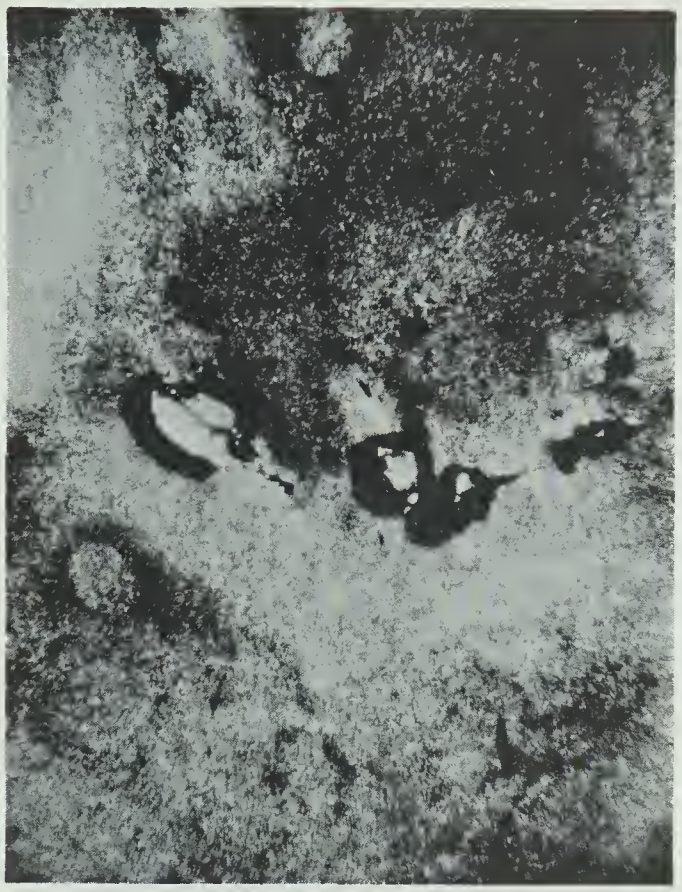


Figure 12

Autoradiograph of a cross section from the epiphyseal region of a femur from a young rat, 3 days after injection of 50 μc of cerium-141. An intense perivascular deposition of the isotope is noted. (Mag. 40X)

Figure 13

Autoradiograph of a longitudinal section from the femur of a young rat injected with 50 μc of cerium-141 and sacrificed 3 days later. Note the deposition of the isotope just below the epiphyseal cartilage plate (a), and the superficial coating on the trabecular network. (Mag. 100X)

may exchange with the cations in the bone crystal itself. Surface adsorption of radiocerium has been previously shown to occur very readily (23). The autoradiographs prepared in this presentation may indicate that the process of radioactive deposition on non-growing bone-crystal surfaces may account for a major portion of cerium-141 incorporation into bone. The autoradiograph in Fig. 13 shows that the radiocerium is not incorporated into the bony trabeculae, but that the darkening appears in the spaces between the spongy network. Magnification of individual bone spicules (Fig. 14) indicates a superficial coating of radioactive material on the surface of the mineralized bone. It is known that in the process of bone growth, trabeculae are constantly being resorbed as they decrease in number to form the secondary spongiosa, or being completely removed to lengthen the bone marrow cavity. The deposition of cerium-141 as indicated by the autoradiographs of Figures 13 and 14 would appear to substantiate Jowsey's theory that radiocerium is localized on areas that are actively resorbing or are already mineralized.

During the growth of cortical bone, continuous remodeling occurs by resorption of bone tissue on the endosteal surface to enlarge the marrow cavity and deposition of bone on the periosteal surface to maintain the thickness of the shaft. Endosteal resorption results in the production of an extensive irregular surface area of highly mineralized bone upon which cerium deposition could readily occur. Autoradiographs showing diaphyseal surfaces (Figs. 15,16) indicate deposition of radiocerium on both periosteal and endosteal sites. Figure 16c

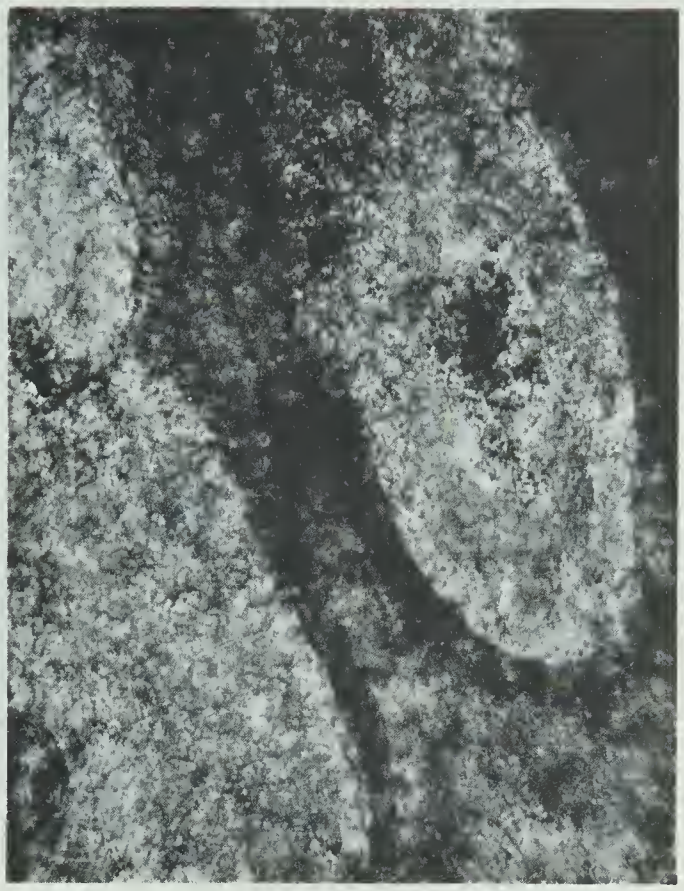


Figure 14

Autoradiograph illustrating an individual bone spicule. Note the deposition of cerium-141 on the bone surface with no apparent incorporation into the bone itself. (Mag. 450X)

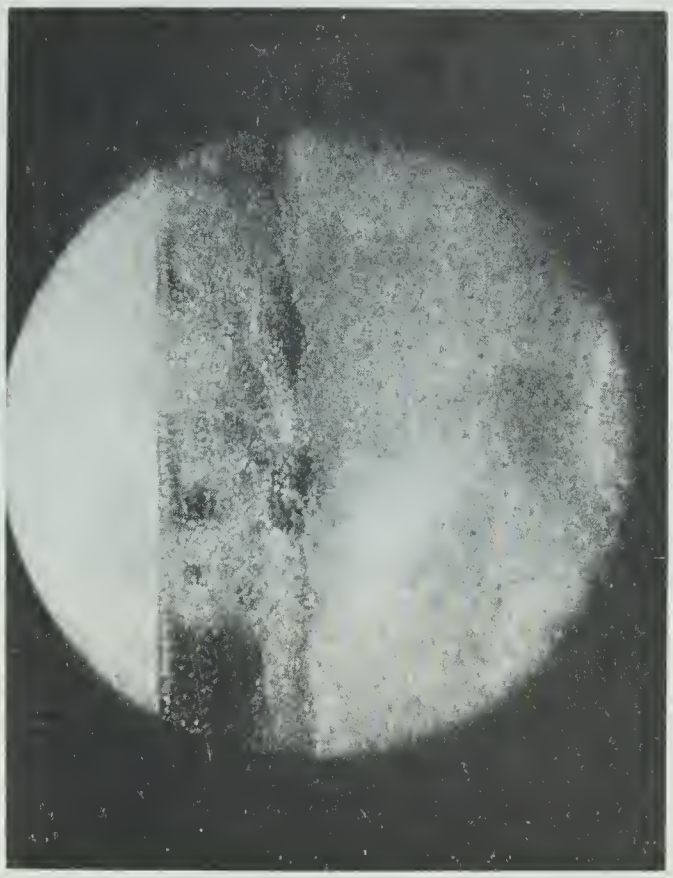


Figure 15

Autoradiograph of a longitudinal section from the femoral diaphysis of a young rat, sacrificed 15 days after injection of 50 μc of cerium-141, indicating periosteal and endosteal radioactive deposition. (Mag. 40X)

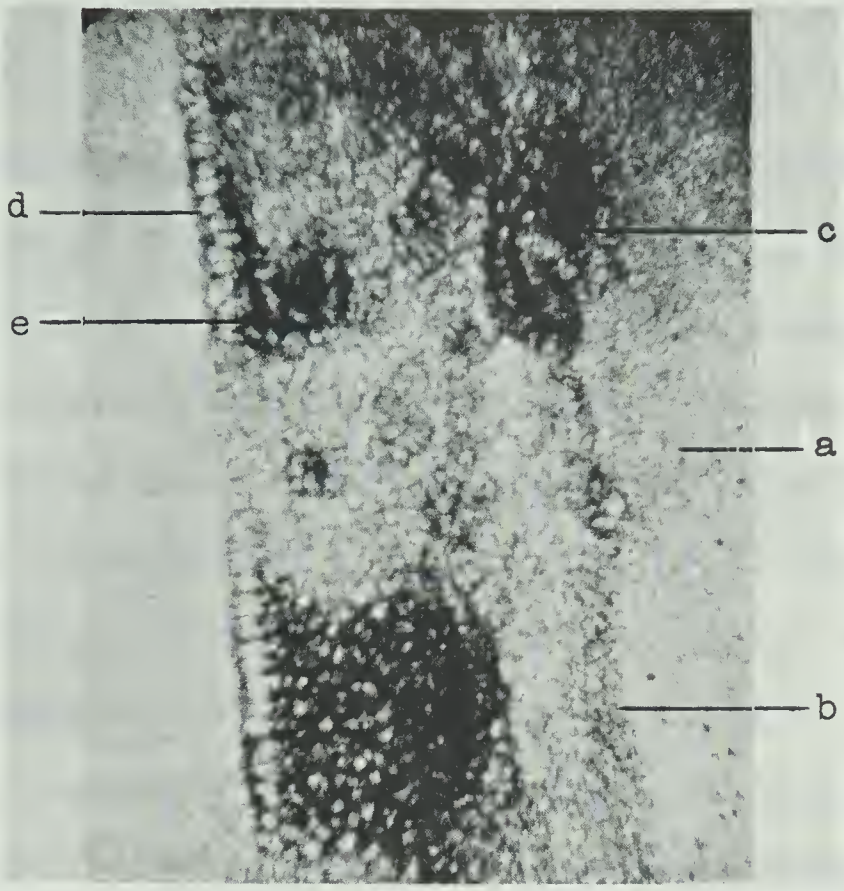


Figure 16

Detailed autoradiographic distribution of radiocerium in the femoral diaphysis of a young rat, 15 days after injection of 50 μc of cerium-141.

a. bone marrow
b. superficial deposition of radiocerium on endosteal surface.
c. local area of high radioactivity on endosteal surface.
d. periosteal deposition of radiocerium.
e. depositions of cerium-141 which have become buried by growth of new bone. (Mag. 100X)

probably represents an area of high osteoclast activity resulting in bone resorption and concurrent intense cerium-141 deposition. The superficial deposition of radiocerium that was noted along the entire border between the endosteum and marrow cavity (Fig. 16b) was likely due to surface adsorption or possible ion exchange with the bone mineral surface.

Localization of radioactivity in the periosteum is interesting since there does not appear to be a continuous surface layer of radioactive deposition (Fig. 15). Upon higher magnification (Fig. 16d), the periosteal deposition of cerium-141 appeared to be around the surfaces of layers of bone cells, presumably osteoblasts, but not incorporated into the bone mineral. It is possible that deposition at these sites may be in the osteoid matrix formed by the osteoblasts. As bone growth continued, these surface depositions of radioactivity likely became buried by new bone (Fig. 16e). It is noteworthy that even 15 days after a single injection of cerium-141, the isotope was still seen as a topical deposition on newly formed bone, indicating that incorporation into the bone shaft proceeds gradually. Since radiocerium was shown to be intensely deposited along vascular complexes in bone (Figs. 10,11,12), it is conceivable that the uneven appearance of radioactivity in the periosteum may be linked with minute blood vessels in the periosteal bone. However, in order to elucidate the exact mechanism of radioactive cerium deposition into bone tissue, a histological study of each section portrayed by the autoradiographs would be necessary to identify such individual structures as osteoblasts, osteoclasts,

uncalcified matrix and newly calcified bone cells with the concurrent deposition of radioactivity in each of these.

Early autoradiographic studies with radioactive isotopes of cerium and other rare earths (6,7,128,129) employed no-screen x-ray films which afforded only limited resolution. Consequently, comparisons of such autoradiographs with histological sections were not able to show the exact localization of the radioactivity. The autoradiographs prepared in this study using Kodak NTB-3 Nuclear Track Plates indicate that satisfactory resolution may be obtained, even on the cellular level, to accurately compare autoradiographs with histological sections by microscopic examination. From the evidence presented by these autoradiographs, it would appear that skeletal incorporation of radiocerium may be due to two processes: deposition on highly mineralized bone surfaces by adsorption or ion exchange, and affinity for uncalcified bone matrix. To further study the deposition of radiocerium in bone, cerium-141 was administered to groups of animals which were maintained on various test diets. Some of the diets were fed to produce conditions resulting in the presence of large excesses of uncalcified bone matrix and others to cause extensive resorption of bone.

4. Effect of Age on the Distribution and Excretion of Cerium-141 in the Rat

A comparison of the levels of cerium-141 in the various organs (Figs. 17,18; Table 5) and excreta from separate groups of young and mature rats, indicated that the young animals retained significantly larger amounts of the isotope in the livers and bone tissues than the mature rats. The older animals,

TABLE 5

Effect of Age on the Distribution and Excretion of Cerium-141 in the Rat[a]

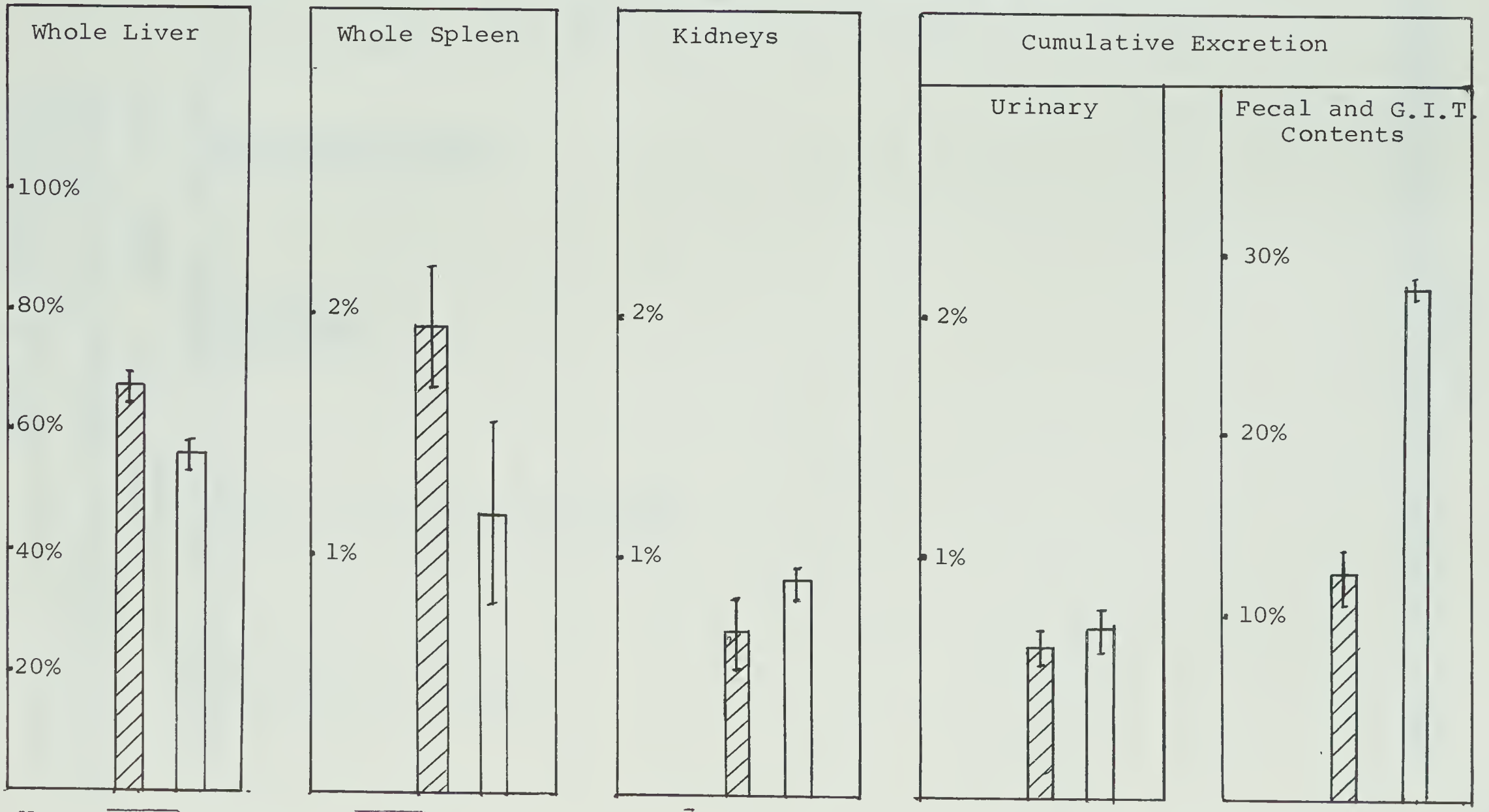
Control Animals	No. of Animals	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
					Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean	6	68.56	1.93	0.68	2.47	1.64	0.60	12.30
S.D.[d]		6.27	0.93	0.09	0.25	0.40	0.14	1.81
S.E.M.[e]		2.56	0.38	0.04	0.10	0.16	0.06	0.74
Test Animals								
Mean	6	56.58	1.16	0.91	1.51	0.79	0.63	28.30
S.D.		5.88	0.50	0.37	0.38	0.26	0.18	2.95
S.E.M.		2.40	0.20	0.15	0.15	0.11	0.07	1.20
Stat. Signif.[f]		P<0.001	NS	NS	P<0.001	P<0.01	NS	P<0.001

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ. For individual values, see Appendix (Table V).
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Standard deviation of the mean.
e. Standard error of the mean.
f. NS indicates that the difference was not significant at the 95 per cent level when compared with control animals.

Figure 17.

Effect of Age on the Distribution and Excretion of Cerium-141 in the Rat

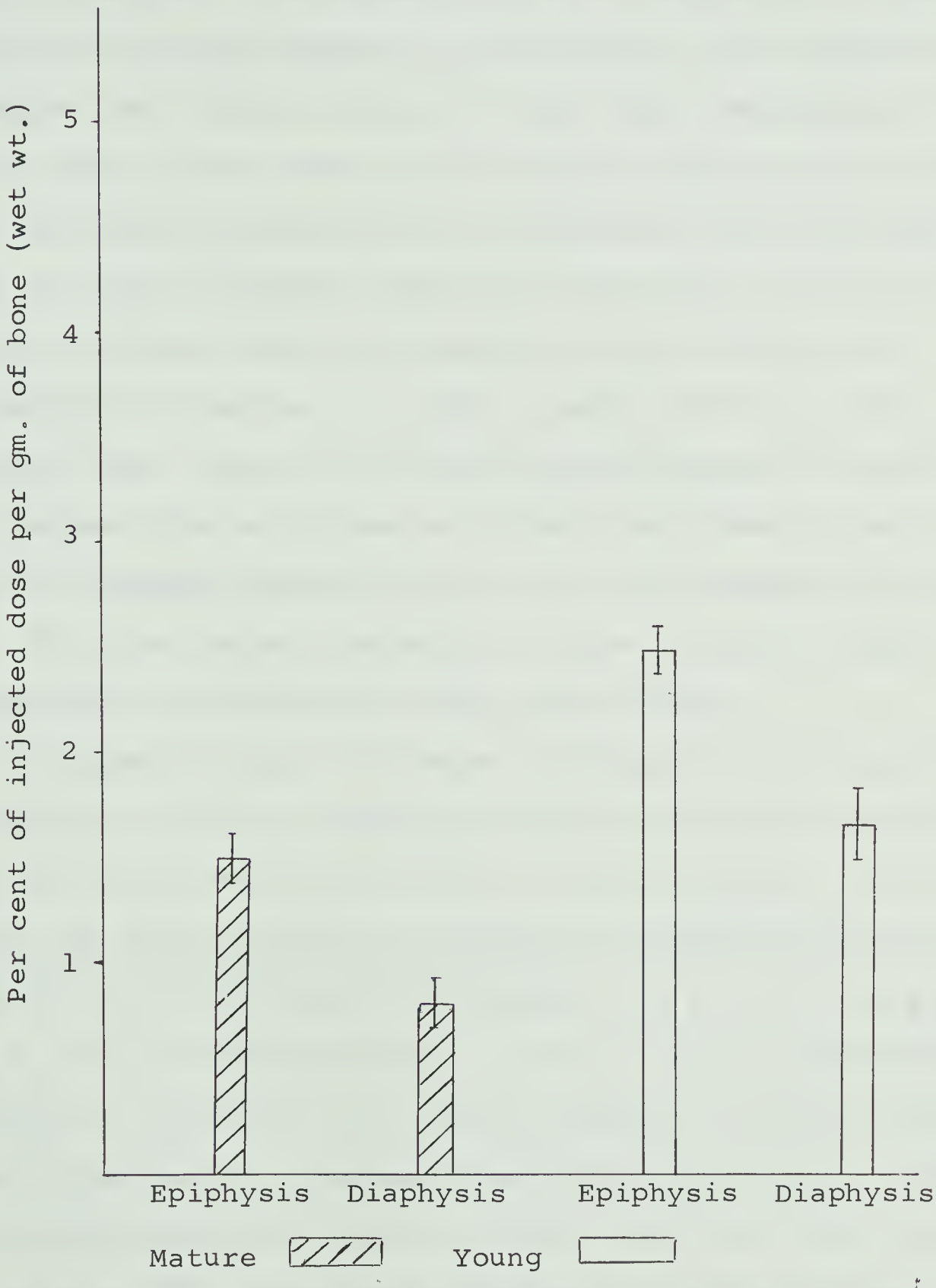
Expressed as per cent of injected dose, 5 days after i.v. injection.



Young ▨ Mature □ Vertical bars] represent the standard error of the mean.

Figure 18.

Effect of Age on the Concentration of Cerium-141 in Bone



Vertical bars ⌶ represent the standard error of the mean.

Animals sacrificed 5 days after i.v. injection of cerium-141, 15 μc/kg.

however, appeared to excrete about twice as much of the radiocerium via the feces as did the young rats. The difference in age did not appear to significantly affect the amount of cerium-141 contained in the kidneys, spleen, or cumulative urinary excretion. The concentrations of cerium-141 per gm. of bone were somewhat higher in the epiphyses and diaphyses of the younger rats than in those of their older counterparts. However, when the per cent of the injected dose retained by the entire skeleton was calculated by determining the difference between the total injected amount and the amount contained in the liver, spleen, kidney and excreta, it was found to be approximately 16 per cent in young animals and 11 per cent in the mature ones. Thus, the outstanding difference in cerium levels between the two groups of animals was shown to be in the amounts of isotope retained by the liver and excreted via the feces. The older animals appeared to have a greater capacity for excreting the radiocerium from their livers.

Considering that the bones in young rats were growing very rapidly, it is not surprising that high skeletal concentrations of radiocerium were noted in these animals. In young animals, the highly vascular epiphyses of endochondral bones as well as the actively resorbing endosteum and proliferating bone cells of the periosteum present available areas of radiocerium deposition as observed in the autoradiographic study performed in this investigation. Because bone growth processes in these areas are not nearly as active in mature rats as in the case of young animals (99), lower concentrations of cerium-141 per gm. of bone were noted in the older rats. It was noteworthy that

the calculated overall skeletal retention of the injected cerium-141 was only slightly lower in the mature rats than in the young animals. These findings agree with those of Copp et al. (128).

Investigations into the effects of age upon skeletal retention of other bone-seeking isotopes such as radioactive calcium or strontium have indicated that young animals concentrate several times as much of these nuclides as do mature ones (128,131). Thus, in contrast to the strikingly large increases reported for the skeletal absorption of radiostrontium and calcium relatively little difference was noted in the skeletal deposition of cerium-141 in young and mature rats. However, it must be mentioned that isotopes of strontium and calcium were reported to be incorporated into the bone salt, whereas radiocerium has been shown to be deposited only superficially on bone matrix and highly calcified bone areas. Bones of mature animals, which have a comparatively slow rate of bone resorption and formation, probably present large areas of highly calcified bone crystals for deposition of radiocerium by adsorption or ion exchange.

5. Effects of a Rachitogenic Diet on the Distribution and Excretion of Cerium-141 in the Rat

A comparison of the distribution of radiocerium in two groups of animals, control rats and those maintained on a rachitogenic diet[a] for 32 days, indicated that the rachitic series concentrated more of the isotope in their bones and

---

[a]Rachitogenic Diet, see page 26.

excreted less of the cerium-141 through the feces than the controls (Table 6; Figs. 19,20). The amounts of radiocerium contained in the liver, spleen, kidneys or cumulative urinary excreta did not differ significantly in the two groups of animals. The per cent of administered cerium-141 retained by the whole skeleton as calculated by the method of difference between the amount administered and that contained in the liver, spleen, kidney and excreta was about 28 per cent for the rachitic and 16 per cent for control animals, respectively.

A rachitogenic diet such as the one used in this investigation when administered to rats, together with a seclusion from ultraviolet light, results in a deficient calcification of the osteoid matrix. This is particularly observed in the newly forming bone at the epiphyses (132,133). As a result, the zone of provisional calcification increases in width and is incompletely mineralized. An irregular growth of soft osteoid tissues follows, and consequently, the trabeculae become surrounded with wide osteoid seams (89). Furthermore, during prolonged deficiency of calcium and phosphate in the body fluids, an increase in parathyroid hormone secretion protects the body against hypocalcemia by causing osteoclast resorption of the bone mineral (134).

Gross examination of the femurs from the rachitic rats in this study showed that the bones were soft and pliable as compared to the white hard bones of the control animals. The marrow, visible through the thin cortex of the rachitic bones, gave them a violet-blue appearance. Bones from the rachitic animals also seemed more vascular, with blood oozing from their

TABLE 6

Effect of a Rachitogenic Diet on the Distribution and Excretion of Cerium-141 in the Rat[a]

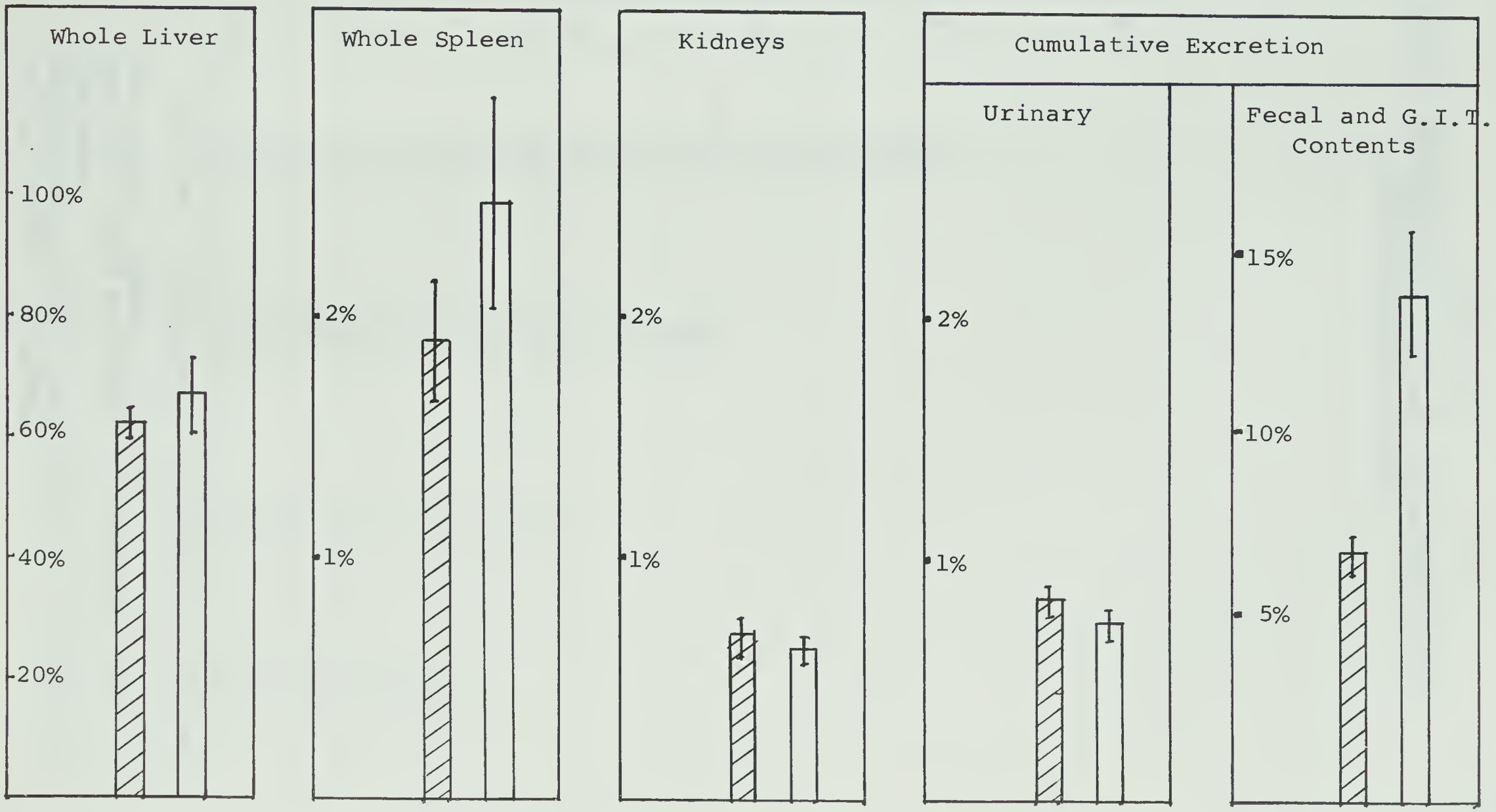
Control Animals	No. of Animals	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
					Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean	4	65.37	1.98	0.63	1.90	1.30	0.74	13.89
S.D.[d]		7.16	0.80	0.05	0.29	0.26	0.08	1.75
S.E.M.[e]		3.58	0.40	0.02	0.15	0.13	0.04	0.88
Test Animals								
Mean	5	65.13	1.92	0.68	4.02	2.74	0.85	6.87
S.D.		3.91	0.47	0.27	0.59	0.52	0.12	1.36
S.E.M.		1.75	0.21	0.12	0.26	0.23	0.05	0.61
Stat. Signif.[f]		NS	NS	NS	P<0.001	P<0.01	NS	P<0.001

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ. For individual values, see Appendix (Table VI).
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Standard deviation of the mean.
e. Standard error of the mean.
f. NS indicates that the difference was not significant at the 95 per cent level when compared with control animals.

Figure 19.

Effect of Rachitogenic Diet on the Metabolism of Cerium-141 in the Rat

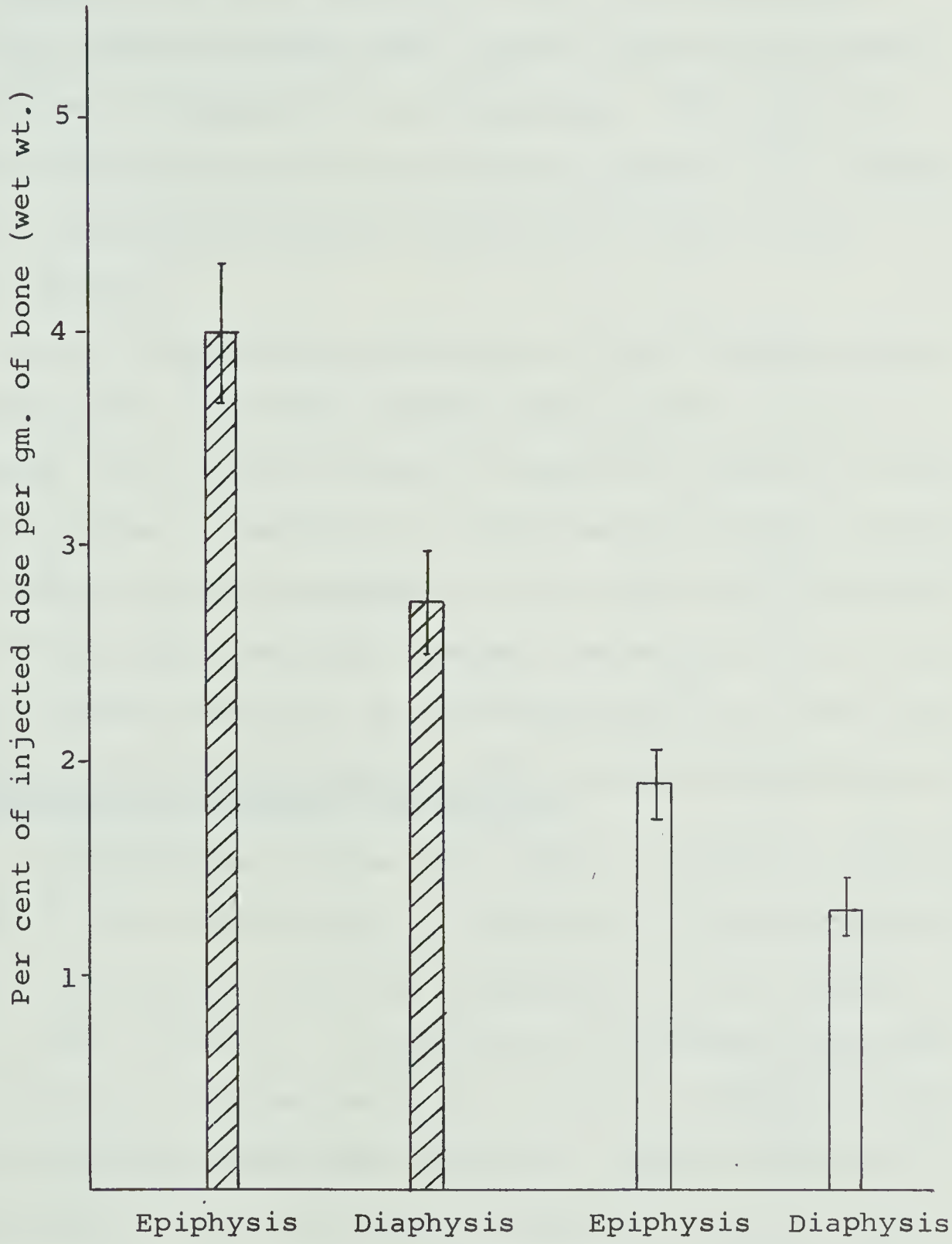
Expressed as per cent of injected dose, 5 days after i.v. injection.



Test ▨ Control □ Vertical bars ⌶ represent the standard error of the mean.

Figure 20.

Effect of Rachitogenic Diet on the Concentration of Cerium-141 in Bone



Test ▨ Control □

Vertical bars] represent the standard error of the mean.

Animals sacrificed 5 days after i.v. injection of cerium-141, 15 μc/kg.

cortex when bent or squeezed.

The increased radiocerium retention by the bones of the rachitic rats may be a result of two factors: the formation of large amounts of uncalcified osteoid matrix for which cerium appears to have an affinity, and the accelerated resorption processes which would expose highly mineralized bone surfaces to deposition by adsorption. The elevated skeletal concentrations of the isotope appeared to be paralleled by a decreased excretion through the feces in the rachitic animals.

It is interesting to note that in the latter animals the very factors which may increase the skeletal uptake of radiocerium were actually shown to reduce the skeletal burden of such isotopes as radioactive strontium and calcium (95,96). Such findings are understandable, since a decreased mineralization of bone would naturally impede the deposition of strontium and calcium which are incorporated mostly into bone salt.

6. Effects of a Phosphorous-Low Diet on the Distribution and Excretion of Cerium-141 in the Rat

A phosphorous-low diet was shown to produce a striking increase in the concentration of cerium-141 in bone tissue as well as a decrease in the fecal elimination of the isotope (Table 7; Figs. 21,22). No significant difference was noted in the uptake of radiocerium by the liver and kidneys of control and phosphorous-deficient animals. Splenic levels of radioactivity in the test animals were only slightly above those in the controls. The estimated skeletal retention of the total administered dose of cerium-141 was approximately 23 per

TABLE 7

Effect of Phosphorous-Low Diet on the Distribution and Excretion of Cerium-141 in the Rat[a]

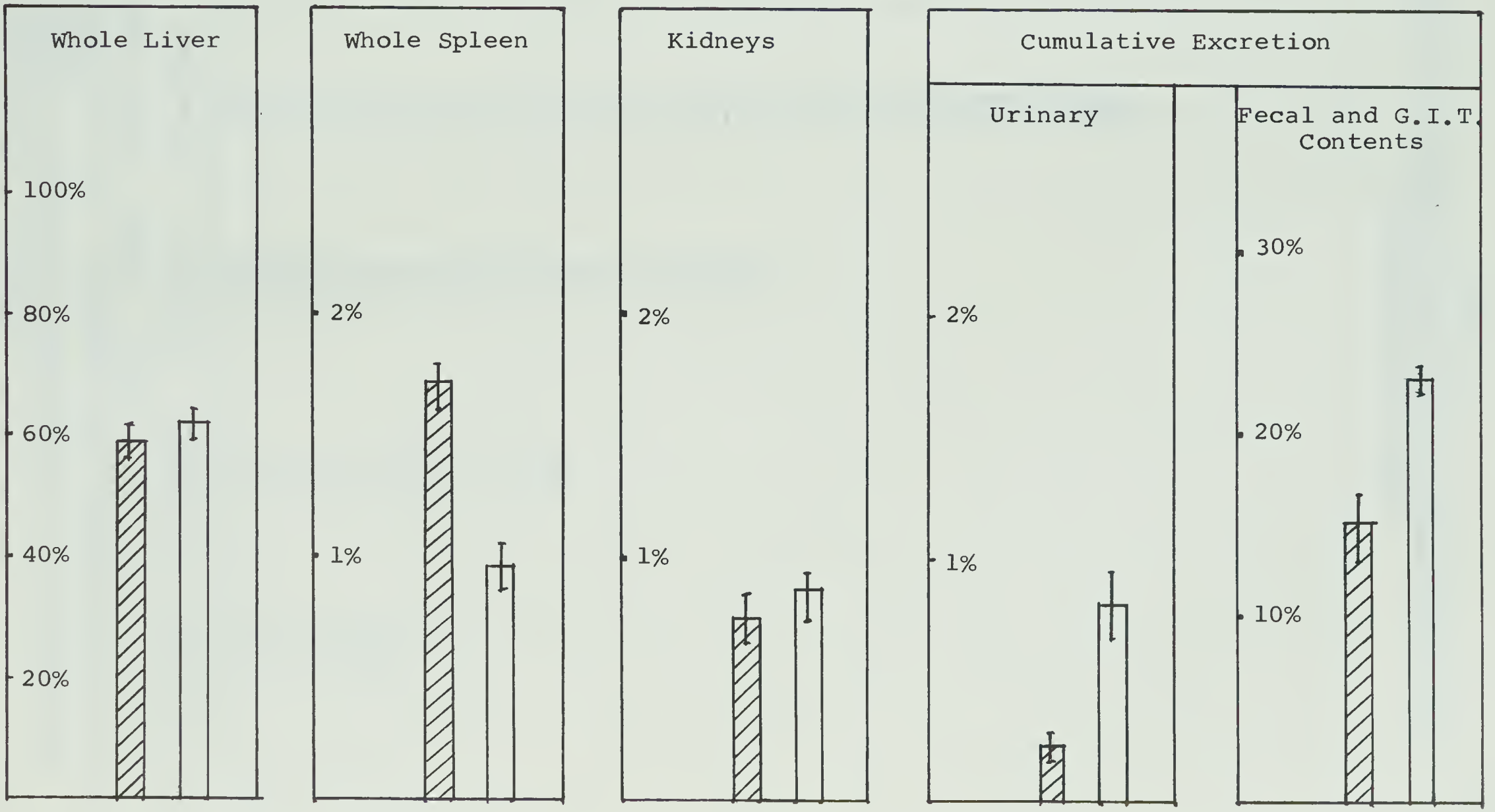
Control Animals	No. of Animals	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
					Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean	4	61.74	0.93	0.87	1.91	1.04	0.60	23.00
S.D.[d]		2.62	0.17	0.21	0.23	0.52	0.27	1.04
S.E.M.[e]		1.31	0.08	0.10	0.11	0.26	0.13	0.52
Test Animals								
Mean	6	58.69	1.63	0.76	5.02	3.00	0.27	15.52
S.D.		4.60	0.30	0.30	0.78	0.66	0.11	3.03
S.E.M.		1.87	0.12	0.12	0.35	0.32	0.04	1.25
Stat. Signif.[f]		NS	P<0.01	NS	P<0.001	P<0.01	NS	P<0.01

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ. For individual values, see Appendix (Table VII).
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Standard deviation of the mean.
e. Standard error of the mean.
f. NS indicates that the difference was not significant at the 95 per cent level when compared with control animals.

Figure 21.

Effect of Phosphorous-Low Diet on the Distribution and Excretion of Cerium-141 in the Rat

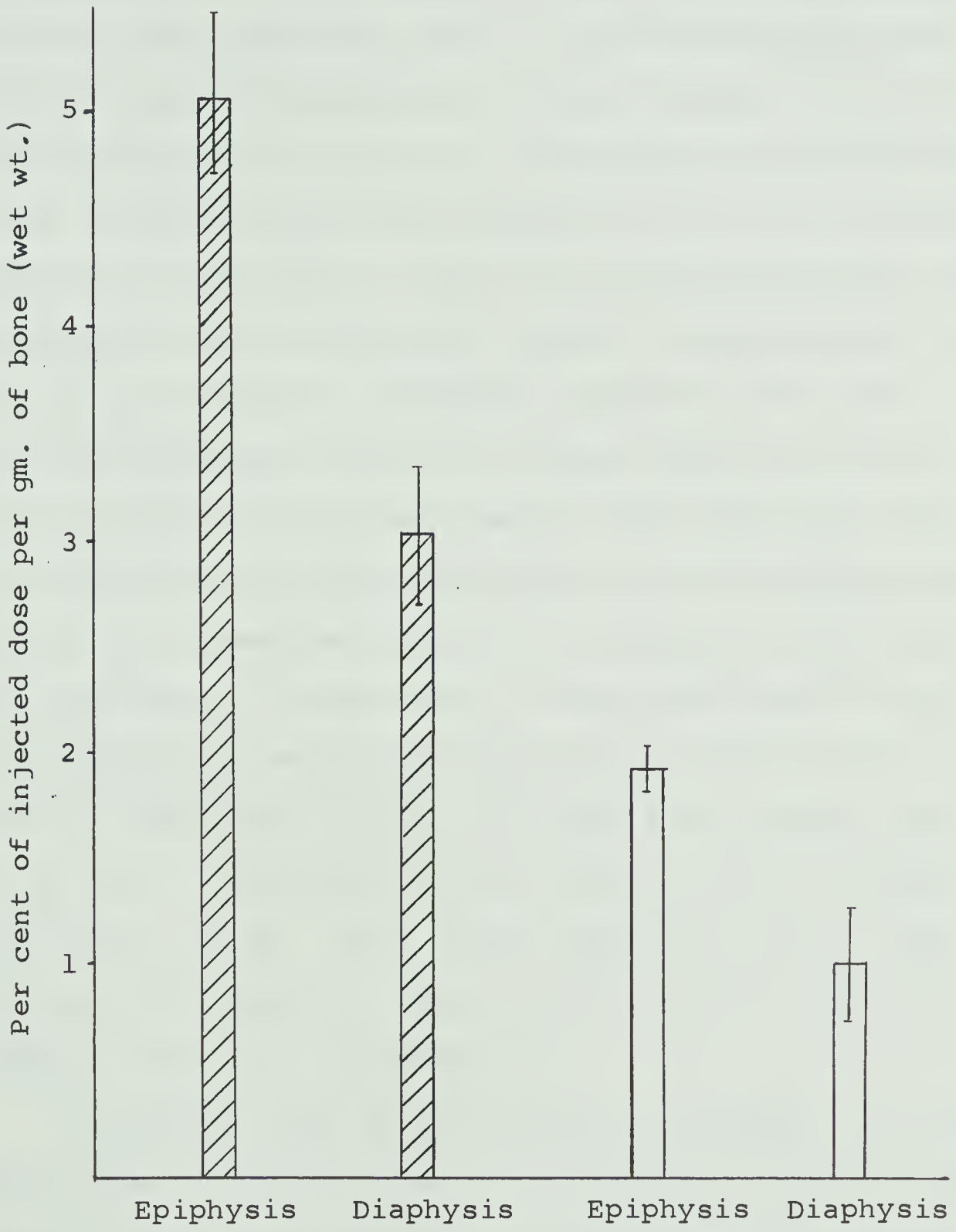
Expressed as per cent of injected dose, 5 days after i.v. injection



Test ▨ Control □ Vertical bars ⌶ represent the standard error of the mean.

Figure 22.

Effect of Phosphorous-Low Diet on the Concentration of Cerium-141 in Bone



Vertical bars I represent the standard error of the mean.
Animals sacrificed 5 days after i.v. injection of cerium-141, 15 μc/kg.

cent in the test animals and only about 13 per cent in the controls.

A diet deficient in phosphorous has been reported to produce a type of rickets in the rat characterized by severe demineralization of the skeleton (135). It was also noted that even while these rats were on a phosphorous-low diet, they still excreted phosphorous and particularly large quantities of calcium via the urine. The skeletal demineralization was ascribed by Day and McCollum (135) to the extensive resorption of bone salt in order to provide phosphorous for the essential needs of the soft tissues. Consequently, the depleting of calcium and phosphate, as well as the bone resorption processes, resulted in bones containing large amounts of uncalcified organic matrix free from bone salt. Thus, the femurs and tibiae from the rats on the phosphorous-deficient diet resembled those of the rachitic animals previously described. In addition, it was noted that the phosphorous deficiency seriously impaired the growth of the animals so that after 32 days, the test rats weighed only about 80 gm., in comparison to the controls which weighed approximately 158 gm. This severe disability of the test animals may have been the cause of the apparent increase in the splenic retention of cerium-141.

As was the case with the rachitic animals, the increased concentration of radiocerium in the skeletons of phosphorous-deficient rats could probably be attributed to the presence of large amounts of uncalcified osteoid matrix in the bones. Large actively resorbing areas would be expected to expose

highly mineralized bone surfaces to deposition of radiocerium by adsorption as well.

The results obtained in this experiment are in agreement with those of Copp et al. (128,129) who compared the effect of phosphorous deficiencies on the retention of radiocerium and radiostrontium. These workers reported that the phosphorous-deficient animals retained a larger amount of radiocerium and excreted less than the control animals. Conversely, these investigators noted that the test animals retained only one-third as much of an administered dose of radioactive strontium and excreted three times as much of this isotope as the controls. Autoradiographic evidence was presented to indicate that the radiocerium administered to a phosphorous-deficient animal was heavily localized in the uncalcified matrix below the epiphyses of long bones. In contrast, the radiostrontium was concentrated in the thin shell of calcified bone remaining in the shaft (128).

7. Effects of a Calcium-Deficient Diet on the Distribution and Excretion of Cerium-141 in the Rat

A calcium-deficient diet appears to have affected only the skeletal retention and fecal elimination of cerium-141 (Table 8; Figs. 23,24). Lack of dietary calcium increased the concentration of radiocerium in bones slightly but significantly and the concurrent decrease in fecal excretion of the isotope was similarly small. No significant difference was noted in the quantities of cerium-141 contained in the liver, spleen, kidneys or urinary excreta of the test and control animals.

A diet deficient only in calcium but adequate in vitamin D and phosphorous has been reported to produce

TABLE 8

Effect of Calcium Deficiency on the Distribution and Excretion of Cerium-141 in the Rat[a]

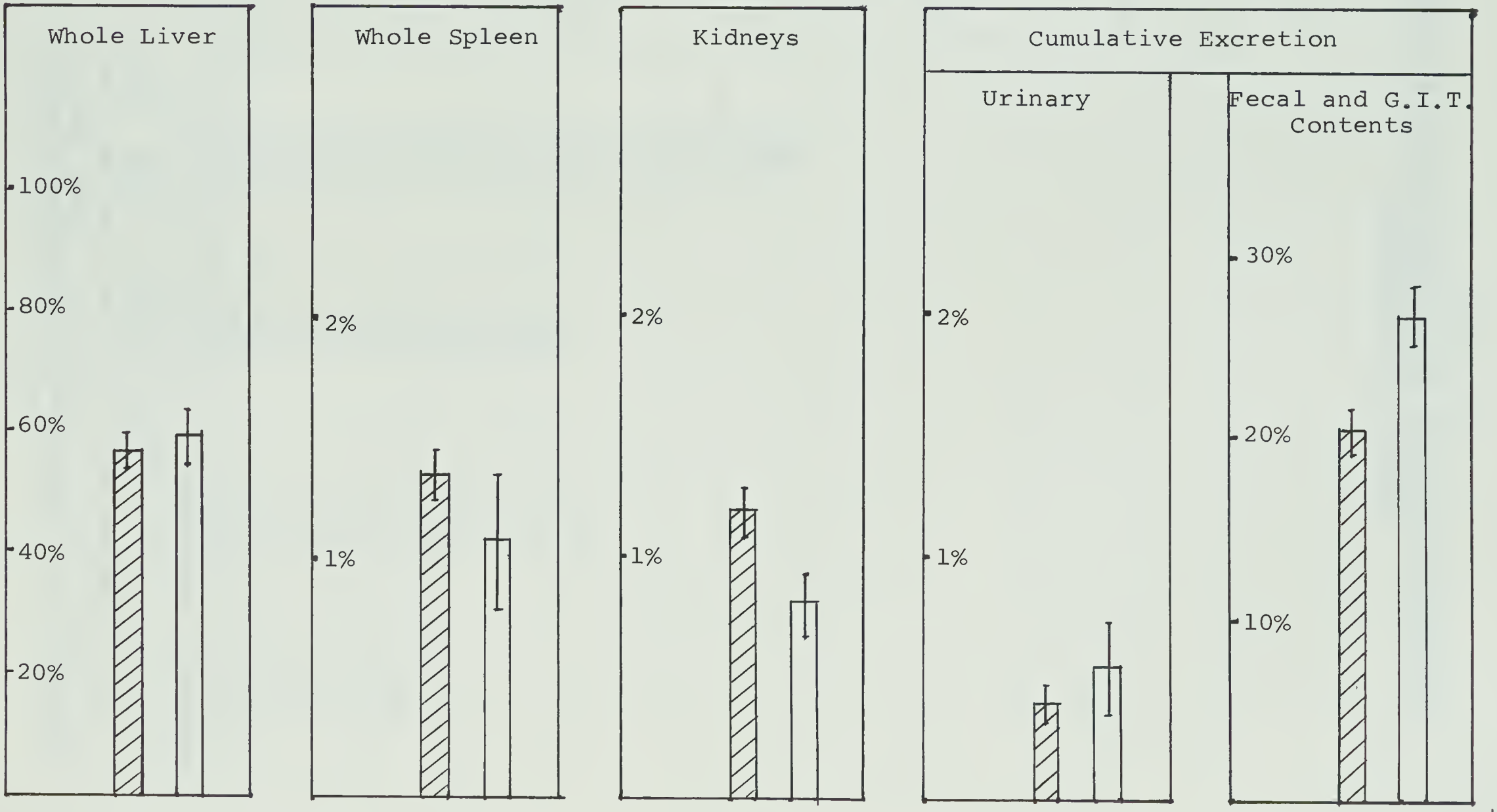
Control Animals	No. of Animals	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
					Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean	4	58.95	1.07	0.81	2.04	1.09	0.54	26.75
S.D.[d]		6.47	0.62	0.22	0.13	0.10	0.38	2.45
S.E.M.[e]		3.24	0.31	0.11	0.65	0.05	0.19	1.22
Test Animals								
Mean	6	56.95	1.29	1.16	3.19	1.87	0.40	20.78
S.D.		3.35	0.23	0.36	0.31	0.24	0.16	3.18
S.E.M.		1.36	0.09	0.14	0.13	0.09	0.06	1.30
Stat. Signif.[f]		NS	NS	NS	$P<0.001$	$P<0.001$	NS	$P<0.05$

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ. For individual values, see Appendix (Table VIII).
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Standard deviation of the mean.
e. Standard error of the mean.
f. NS indicates that the difference was not significant at the 95 per cent level when compared with control animals.

Figure 23.

Effect of Calcium Deficiency on the Distribution and Excretion of Cerium-141 in the Rat

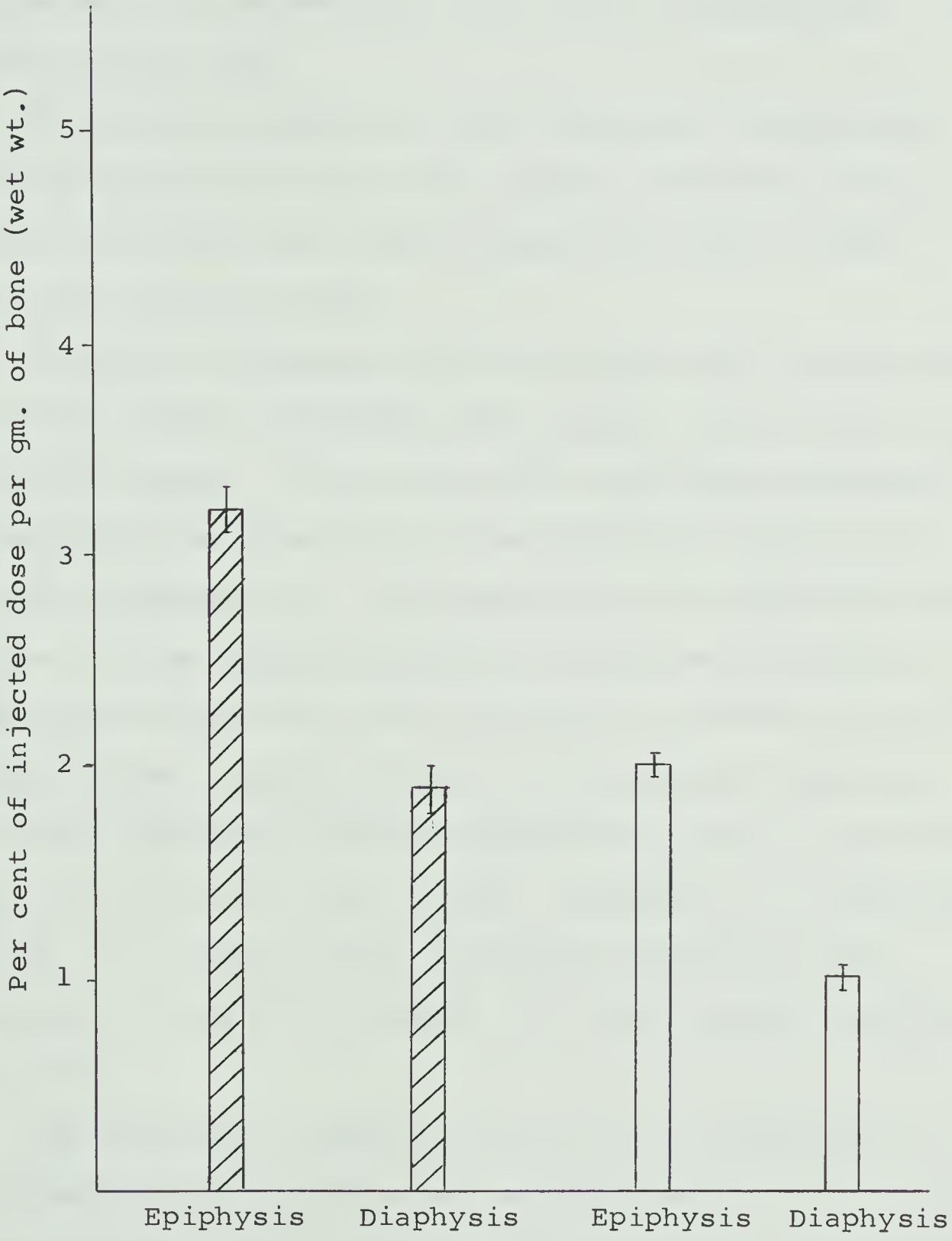
Expressed as per cent of injected dose, 5 days after i.v. injection



Test ▨ Control □ Vertical bars ⌶ represent the standard error of the mean.

Figure 24.

Effect of Calcium Deficiency on the Concentration of Cerium-141 in Bone



Vertical bars ⟊ represent the standard error of the mean.
Animals sacrificed 5 days after i.v. injection of cerium-141, 15 μc/kg.

osteoporosis, or bone atrophy in rats (89). This condition was characterized by a reduction in the total amount of bone salt within the skeleton. However, the ossification was complete and there was no excess of uncalcified osteoid matrix as seen in the rachitic rats and the phosphorous deficient animals (89).

Upon gross examination, the femurs and tibiae from the calcium-deficient rats in the present study were very brittle. Such bones were white, opaque and hard, in contrast to the rachitic bones.

Harrison and Frazer (89) have shown that the skeletons of rats with calcium deficiency were laying down new bone more rapidly than normal. In spite of such rapid bone formation, bone resorption was proceeding at an abnormally rapid rate resulting in osteoporosis. The mechanism for increased bone resorption in the calcium-deficient animals was ascribed to high parathyroid activity which resulted in the mobilization of calcium stores already present in the animal's skeleton. Some of this mobilized calcium was then utilized in calcification of the regions of rapid growth. Therefore, as the skeleton shared its calcium stores throughout a progressively larger mass of bone during growth, the bones became continually thinner (79).

The increase in skeletal retention of radiocerium in the calcium-deficient animals may be explained by radioactive adsorption on the large areas of resorbing bone. Since there were no great excesses of uncalcified osteoid tissue in these animals, the most likely mechanism of skeletal cerium-141

incorporation was through surface deposition by adsorption onto such resorbing areas. It is interesting to note that the increase in skeletal retention of cerium-141 in the calcium-deficient animals was considerably less than the enhanced radiocerium concentration noted in the rachitic and phosphorous-low animals. In the latter two series, radiocerium incorporation was attributed to two processes: adsorption and affinity for uncalcified osteoid. However, in the calcium-deficient series, cerium-141 skeletal uptake was probably by surface deposition only.

Recently, calcium deficiency in rats was reported to impair the oxidative and reductive metabolic processes in liver cell microsomes (136). Since cerium is known to enter the microsomal fraction of liver cells (8), future studies of cerium metabolism may be indicated to determine whether calcium-deficient rats may be more susceptible to cerium-induced fatty liver.

8. Effects of Vitamin-A-Deficient Diet on the Distribution and Excretion of Cerium-141 in the Rat

A dietary deficiency of vitamin A resulted in an increased retention of the administered radiocerium by the liver of the test animals (Table 9, Fig. 25). A small, but significant increase in the concentration of radiocerium was noted also in the bone samples (Fig. 26). The prolonged liver retention and elevated bone concentration of cerium-141 in the vitamin A-deficient animals was reflected by a greatly lowered fecal excretion of the isotope. No significant changes were noted in the uptake by the kidneys or spleen.

TABLE 9

Effect of Vitamin A-Deficient Diet on the Distribution and Excretion of Cerium-141 in the Rat[a]

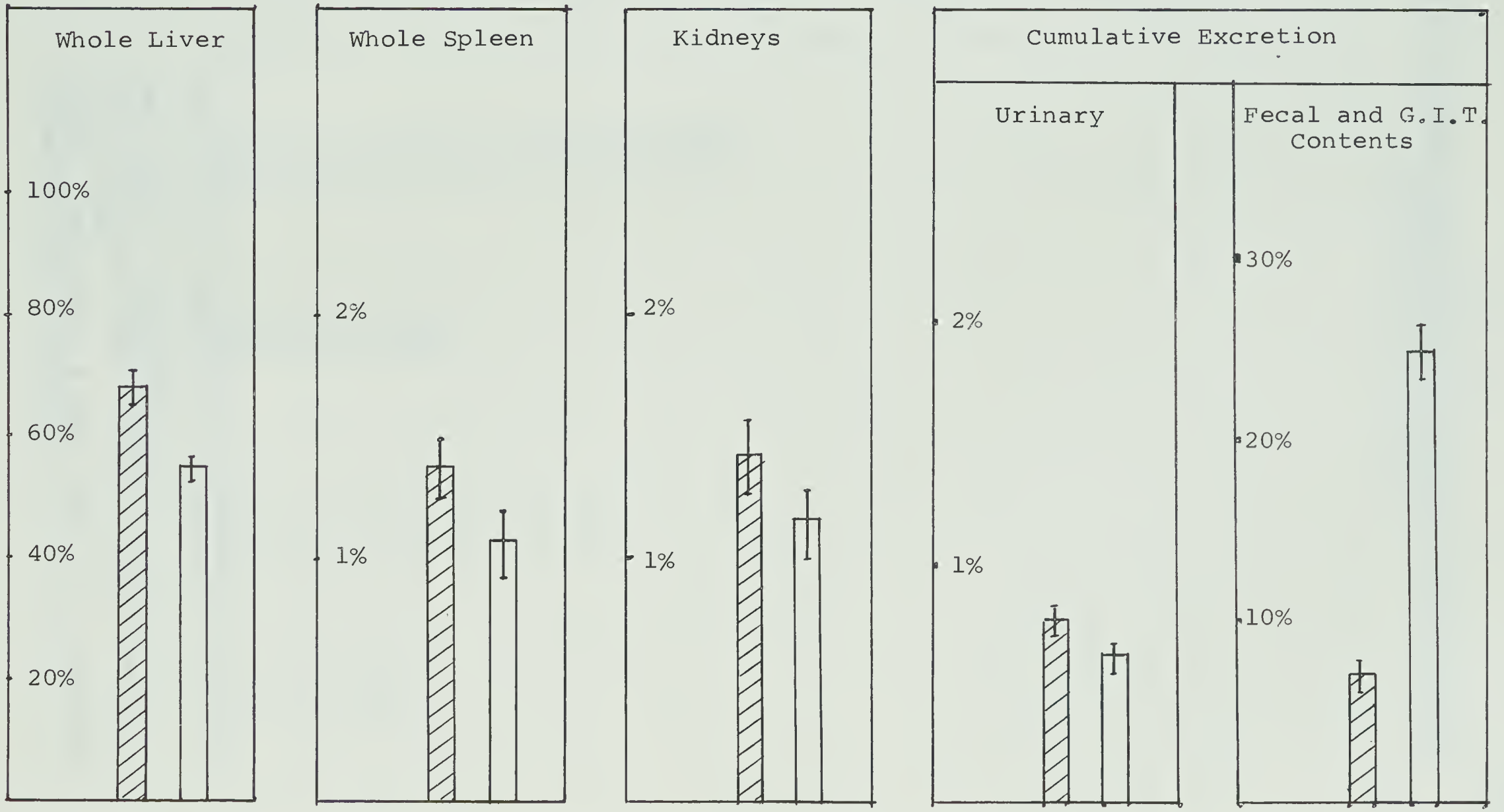
Control Animals	No. of Animals	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
					Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean	5	54.92	1.09	1.15	1.99	0.89	0.64	24.94
S.D.[d]		3.21	0.22	0.30	0.34	0.17	0.10	3.54
S.E.M.[e]		1.31	0.09	0.12	0.14	0.07	0.04	1.44
Test Animals								
Mean	7	66.82	1.36	1.41	2.77	1.22	0.74	7.75
S.D.		4.74	0.24	0.42	0.34	0.23	0.18	2.13
S.E.M.		1.80	0.09	0.16	0.13	0.09	0.07	0.81
Stat. Signif.[f]		P<0.01	NS	NS	P<0.01	P<0.05	NS	P<0.001

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ. For individual values, see Appendix (Table IX).
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Standard deviation of the mean.
e. Standard error of the mean.
f. NS indicates that the difference was not significant at the 95 per cent level when compared with control animals.

Figure 25.

Effect of Vitamin A-Deficient Diet on the Distribution and Excretion of Cerium-141 in the Rat

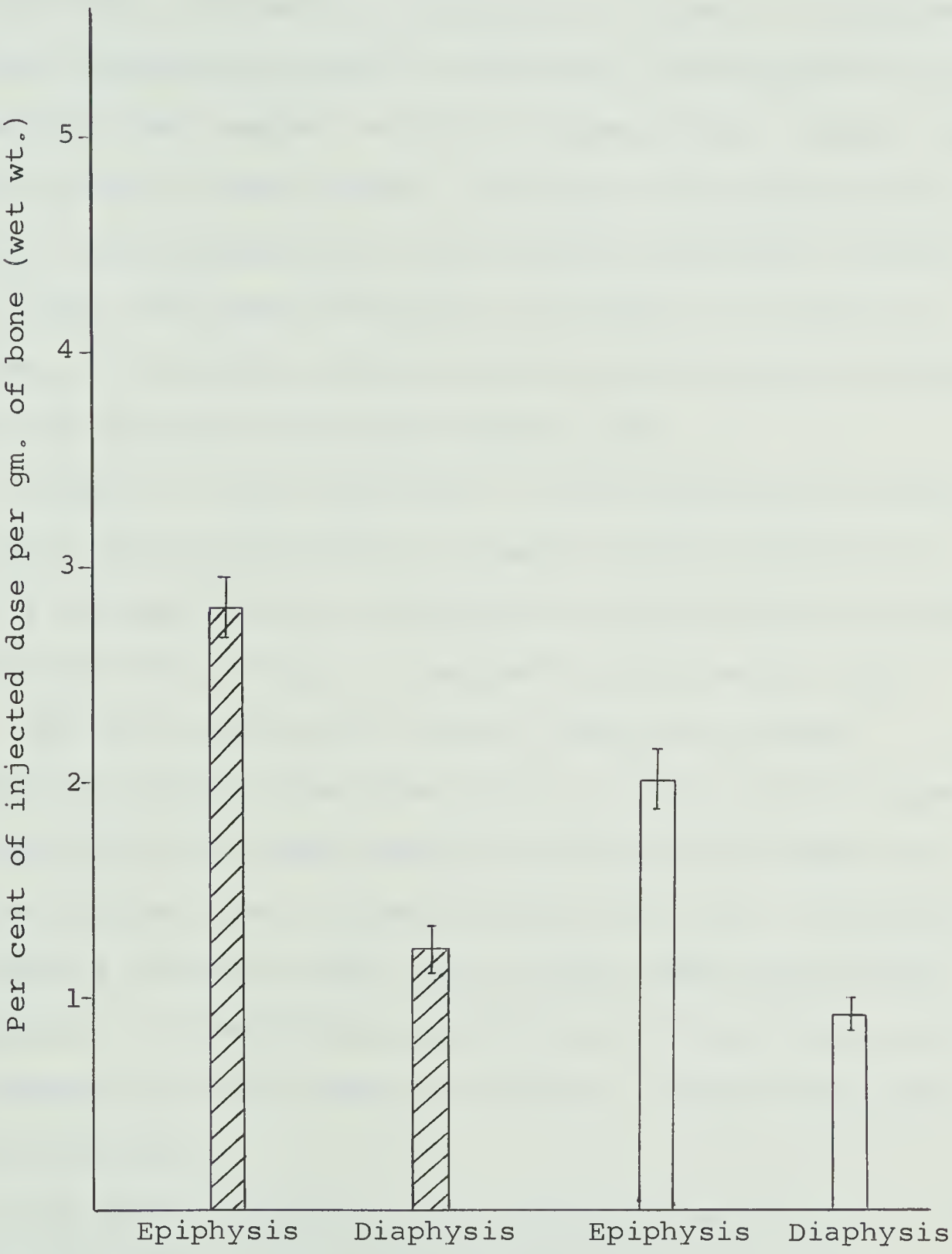
Expressed as per cent of injected dose, 5 days after i.v. injection



Test ▨ Control ▭ Vertical bars I represent the standard error of the mean.

Figure 26.

Effect of Vitamin A-Deficient Diet on the Concentration of Cerium-141 in Bone



Vertical bars I represent the standard error of the mean.
Animals sacrificed 5 days after i.v. injection of cerium-141, 15 μc/kg.

Lack of vitamin A, as manifested in the bone metabolism of rats, varies with the severity of the deficiency. In very young growing animals, vitamin A deficiency has been shown to interfere drastically with the growth pattern of bones (137,138). In severe shortages of vitamin A, epiphyseal cartilage sequences of growth, maturation and degeneration were retarded and even stopped entirely in some cases. Hence, longitudinal growth of bone ceased. Although the resorption of trabecular and endosteal bone was greatly diminished, periosteal growth continued throughout the stages of vitamin A deficiency. The overall effect was the production of thicker, coarser and shorter-than-normal bones (137).

However, it was noted that in rats which were reared on a normal diet to the age of 3 weeks and then placed on a vitamin A-deficient diet, endochondral ossification was almost normal except for the great overgrowth of periosteal bone (139). The rats used in the present study very likely resembled the animals described above. In the present experiment, the young rats were weaned at the age of 24 days and subsequently placed on a vitamin A-deficient diet. Because of the vitamin A stores in the liver, a very severe deficiency was probably not created during the period of test diet. No gross xerophthalmia was noted but several animals developed corneal ulceration.

The small increase in the skeletal deposition of radiocerium in the vitamin A-deficient rats was probably caused by the great overgrowth of periosteal bone. Deposits of cerium-141 on the periosteum were likely buried by a

continuous, rapid formation of new bone.

In the vitamin A-deficient series, the elevated liver retention and reduced elimination of cerium-141 indicated that such a dietary deficiency may have impaired the ability of the liver to excrete the isotope. In the rat, lack of vitamin A has been reported to cause thickening and metaplasia of the endothelial lining of the intrahepatic and extrahepatic bile ducts (140). Such degenerative lesions were followed by production of free masses of cells and debris which accumulated, causing bile stasis and dilatation of the bile ducts. Since elimination via the bile has been shown to be an important route for excretion of radiocerium from the liver (101,53), obstruction of biliary flow in the vitamin A-deficient animals could well be responsible for the elevation of radiocerium in the liver and the concurrent decrease in fecal excretion of the isotope.

In addition, vitamin A-deficient rats were also noted to develop hyperplasia of the reticuloendothelial system, whereby the Kupffer cells of the liver were increased in both size and number (141). Such changes in liver cells may also have contributed to the prolonged hepatic retention and slow excretion of cerium-141 from the liver since it had been previously noted that the reticuloendothelial system of the liver was involved in the concentration of radiocerium (8,20).

9. Effects of Calcium-EDTA on the Distribution and Excretion of Cerium-141 in Normal and Calcium-Deficient Rats

Administration of calcium-EDTA[a] for 4 consecutive days,

[a]Brand of calcium disodium salt of ethylenediamine tetra-acetic acid; Riker Pharmaceutical Co. Ltd.,Toronto,Canada.

starting 24 hours after an injection of cerium-141, resulted in a slight decrease in the liver levels of the radiocerium and a large increase in the urinary excretion of the isotope in both calcium-deficient and in normal animals (Tables 10, 11; Figs. 27,28,29,30). No significant difference was noted in the levels of radiocerium in the bones, spleen, or fecal excretion.

In previous reports concerning the elimination of radiocerium from soft tissues, especially liver and kidneys, by EDTA (110,111,112), the chelating agent was administered simultaneously with the radioactive cerium. Hence it was difficult to ascertain whether the EDTA actually mobilized the isotope from these tissues or if this complexing agent merely prevented the deposition of radiocerium. In the present study, EDTA was administered 1 day after the cerium-141 injection in order to investigate the removal of radiocerium already deposited.

Catsch (112) reported that when EDTA was injected simultaneously with radiocerium, the concentration of the isotope in the liver dropped from 46 per cent to 2 per cent of the injected dose, and in the kidneys from 1.83 per cent to 0.29 per cent of the initial dose. However, in the present study, where EDTA was administered after the actual deposition of cerium-141 had occurred, only a very small reduction in hepatic retention was noted. The slight increase in kidney concentrations was probably due to the increased urinary excretion of the isotope.

Catsch (110,112) also noted that concurrent injections

TABLE 10

Effect of Calcium-EDTA on the Distribution and Excretion of Cerium-141 in Normal Rats[a]

Control Animals	No. of Animals	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
					Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean	4	58.95	1.07	0.81	2.04	1.09	0.54	26.75
S.D.[d]		6.47	0.62	0.22	0.13	0.10	0.38	2.45
S.E.M.[e]		3.23	0.31	0.11	0.65	0.05	0.19	1.22
Test Animals								
Mean	4	52.10	1.59	1.09	2.21	0.98	2.18	27.49
S.D.		4.56	0.71	0.14	0.47	0.26	0.25	2.40
S.E.M.		1.28	0.35	0.07	0.23	0.13	0.12	1.20
Stat. Signif.[f]		P<0.05	NS	NS	NS	NS	P<0.001	NS

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ. For individual values. see Appendix (Table X).
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Standard deviation of the mean.
e. Standard error of the mean.
f. NS indicates that the difference was not significant at the 95 per cent level when compared with control animals.

TABLE 11

Effect of Calcium-EDTA on the Distribution and Excretion of Cerium-141 in Calcium-Deficient Rats[a]

Control Animals	No. of Animals	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
					Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Mean	6	56.95	1.29	1.16	3.19	1.87	0.40	20.78
S.D.[d]		3.35	0.23	0.36	0.31	0.24	0.16	3.18
S.E.M.[e]		1.37	0.09	0.15	0.13	0.09	0.06	1.30
Test Animals								
Mean	5	51.41	1.13	1.29	2.88	1.67	2.61	23.73
S.D.		4.96	0.40	0.15	0.27	0.14	0.49	4.26
S.E.M.		2.21	0.18	0.06	0.12	0.06	0.22	1.90
Stat. Signif.[f]		NS	NS	NS	NS	NS	P<0.001	NS

a. Animals sacrificed 5 days after i.v. injection of 15 µc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ. For individual values, see Appendix (Table XI).
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Standard deviation of the mean.
e. Standard error of the mean.
f. NS indicates that the difference was not significant at the 95 per cent level when compared with control animals.

Figure 27.

Effect of Calcium-EDTA on the Distribution and Excretion of Cerium-141 in Calcium-Deficient Rats

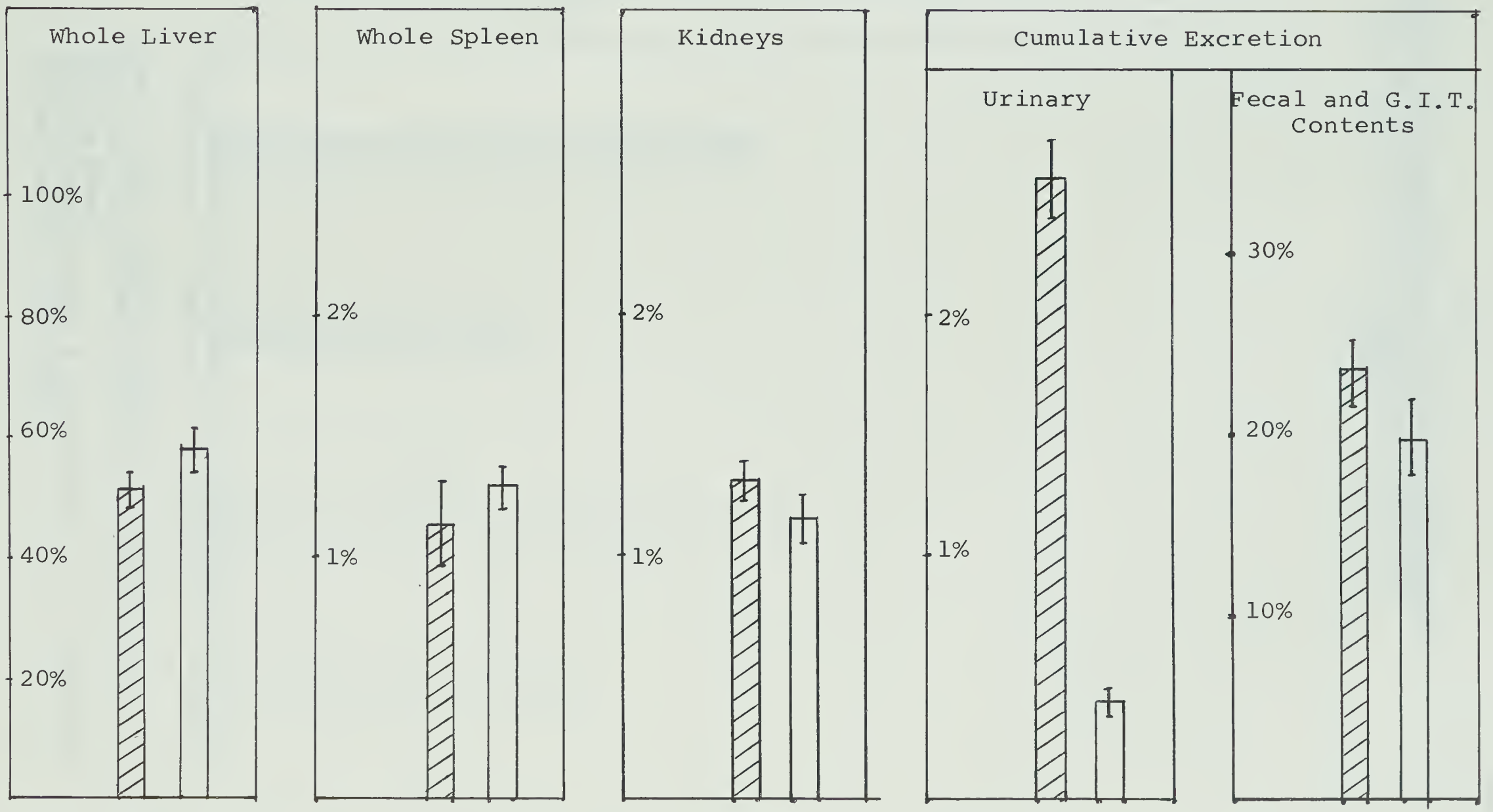
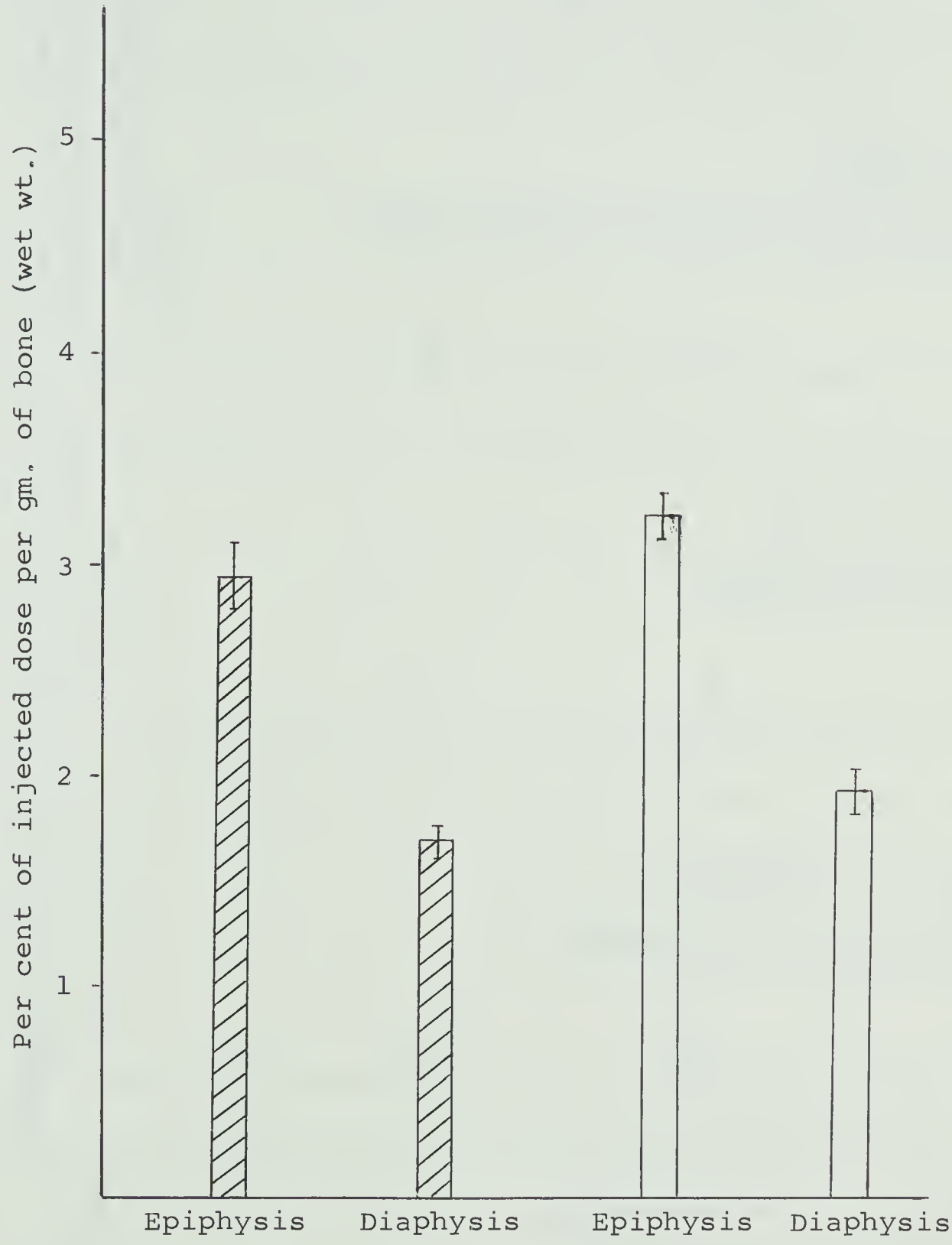


Figure 28.

Effect of Calcium-EDTA on the Concentration of Cerium-141 in Bone of Calcium-Deficient Rats



Test ▨ Control □
Vertical bars ⌶ represent the standard error of the mean.
Animals sacrificed 5 days after i.v. injection of cerium-141, 15 μc/kg.

Figure 29.

Effect of Calcium-EDTA on the Distribution and Excretion of Cerium-141 in the Normal Rat

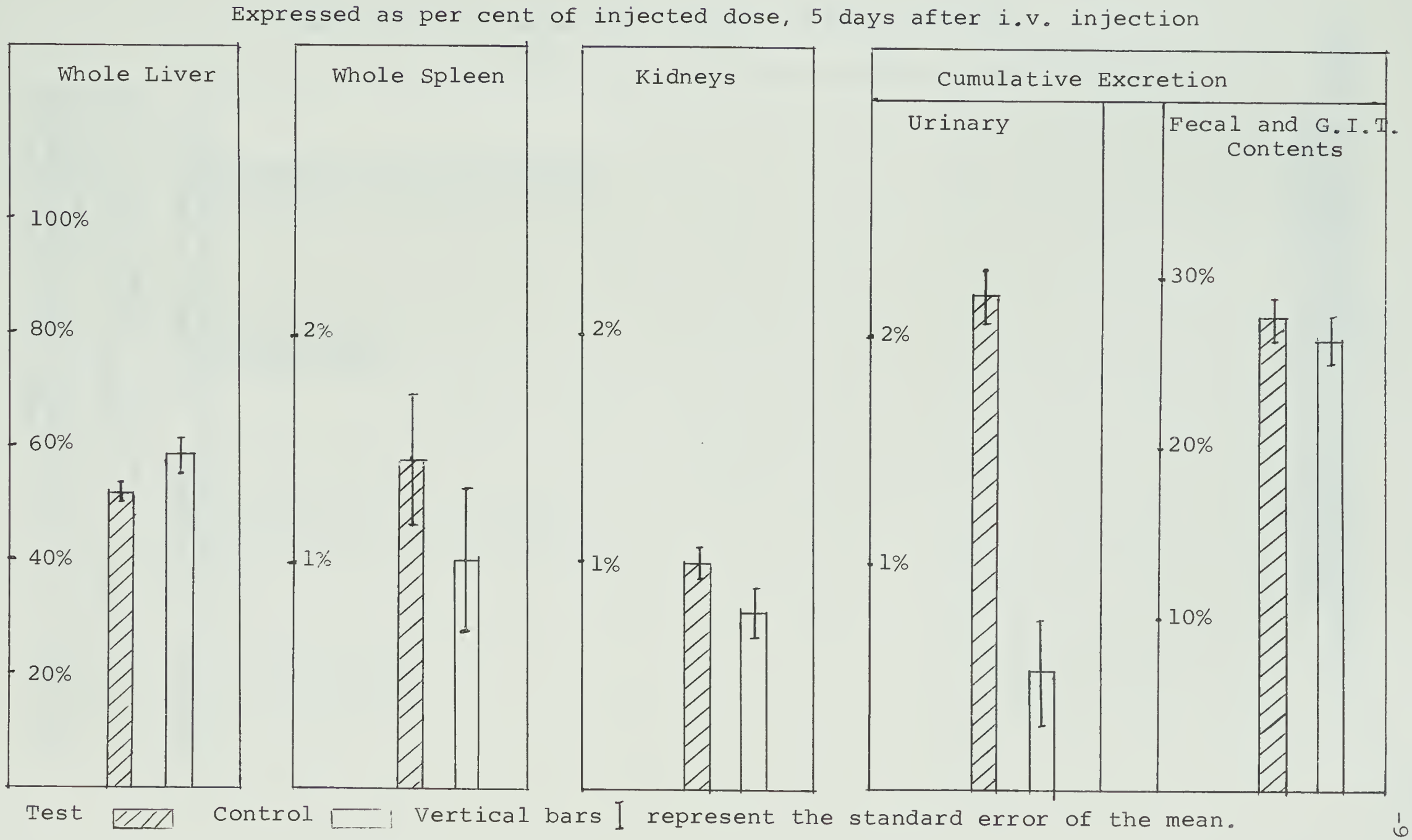


Figure 30.

Effect of Calcium-EDTA on the Concentration of Cerium-141 in Bone of Normal Rats

Test ▨ Control □

Vertical bars ⊥ represent the standard error of the mean.

Animals sacrificed 5 days after i.v. injection of cerium-141, 15 µc/kg.

of radiocerium and EDTA resulted in a slight but significant increase in the skeletal concentration of the isotope. This increase was attributed to the ability of the EDTA-radiometal chelate to diffuse into the extracapillary space, thus bringing enhanced amounts of the radiometal in contact with the ion-exchanging or adsorbing structures of bone. In the present study, where a large portion of the cerium-141 was already deposited in the skeleton after 1 day, subsequent administration of EDTA did not effect any significant changes.

SUMMARY AND CONCLUSIONS

(1) Various tissues of the rat were analyzed for content of cerium-141 after an intravenous administration of cerium-141 citrate complex.

(2) One day after injection, almost the entire administered amount of cerium-141 was distributed throughout the following: liver, spleen, kidneys, skeleton and excreta (including gastrointestinal contents). The liver and bones contained the highest levels of radiocerium. Tissues from the skeletal muscle, prostate, testes, seminal vesicles, brain, heart, lung and thyroids did not show any specific uptake of the isotope.

(3) The distribution and excretion of cerium-141 was studied at intervals of 1, 3, 5 and 21 days after intravenous administration.

Cerium-141 was rapidly concentrated by the liver, where a biological half-life of 15 days was observed for this isotope. Uptake by the bones proceeded gradually, with cerium-141 incorporation into the bone shafts still increasing after 21 days. Excretion of cerium-141 was almost entirely via the fecal route.

(4) An autoradiographic localization of radiocerium presented evidence that, in long bones, cerium-141 was deposited in the zones of active growth beneath the epiphysis, on endosteal and periosteal surfaces, and perivascularly throughout compact bone.

(5) A study on the effect of age on the metabolism of cerium-141 indicated that higher concentrations of the isotope accumulated in the bones and livers of young rats than in those of older, mature animals.

(6) The distribution and excretion of cerium-141 in normal animals were compared with those of animals which were maintained on the following test diets: rachitogenic, vitamin A-deficient, calcium-deficient, and phosphorous-deficient.

(7) All of these test diets resulted in elevated concentrations of cerium-141 in the bone. In addition, deficiency of vitamin A also produced increased hepatic retention of the radiocerium. The mechanisms by which these various test diets may have altered the metabolism of radiocerium were discussed.

(8) Calcium disodium versenate* was ineffective in removing skeletal depositions of cerium-141. The reduction in hepatic levels of radiocerium by this chelating agent was very slight.

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*Brand of calcium disodium salt of ethylenediamine tetra-acetic acid; Riker Pharmaceutical Co. Ltd., Toronto, Canada.

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APPENDIX

TABLE I

Distribution and Excretion of Cerium-141 One Day After Intravenous Injection[a]

Animal No.	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
1	88.06	2.77	0.53	1.93	1.31	0.53	4.98
2	93.46	2.88	0.58	1.35	1.23	0.48	1.46
3	84.55	2.53	0.43	2.16	1.49	0.34	3.65
4	89.70	3.28	0.54	1.75	1.21	0.29	4.84
5	76.51	3.95	0.60	1.03	0.86	0.56	3.02
Mean	86.45	3.08	0.54	1.64	1.22	0.44	3.59

a. Cerium-141 administered as a citrate complex, 15 μc/kg.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.

TABLE II

Distribution and Excretion of Cerium-141 Three Days After Intravenous Injection[a]

Animal No.	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
1	52.75	2.11	0.67	2.20	2.16	0.46	21.36
2	76.10	2.79	0.31	1.28	0.64	0.86	12.66
3	66.50	2.39	0.46	2.16	1.25	0.59	7.68
4	80.36	- [d]	0.61	3.46	2.45	0.66	7.88
5	73.56	0.63	0.92	1.92	0.96	0.38	8.32
6	75.65	2.09	0.84	0.96	0.72	0.27	13.50
Mean	70.82	1.80	0.63	1.99	1.36	0.54	11.89

a. Cerium-141 administered as a citrate complex, 15 μc/kg.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Lost sample.

TABLE III

Distribution and Excretion of Cerium-141 Five Days After Intravenous Injection[a]

Animal No.	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
1	68.64	1.41	0.70	2.49	1.38	0.70	15.94
2	62.56	2.00	0.52	2.55	2.13	0.46	17.42
3	77.15	2.75	0.64	2.86	1.04	0.50	17.19
4	64.26	1.97	1.05	2.91	1.86	0.73	18.25
5	63.55	1.58	0.98	2.10	1.39	0.32	13.13
6	75.42	3.21	0.54	1.54	0.94	0.72	12.93
7	60.08	0.74	0.42	1.89	1.02	0.24	9.82
Mean	67.38	1.95	0.69	2.33	1.39	0.52	14.95

a. Cerium-141 administered as a citrate complex, 15 μc/kg.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.

TABLE IV

Distribution and Excretion of Cerium-141 Twenty-One Days After Intravenous Injection[a]

Animal No.	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
1	35.67	1.22	0.91	1.72	1.56	0.64	42.30
2	32.91	2.00	1.51	2.60	1.80	1.76	38.32
3	28.30	0.84	1.09	2.10	1.66	1.20	44.06
Mean	32.29	1.35	1.17	2.14	1.67	1.21	41.56

a. Cerium-141 administered as a citrate complex, 15 μc/kg.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.

TABLE V

Effect of Age on the Distribution and Excretion of Cerium-141 in the Rat[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Young Rat[d]	68.64	1.41	0.70	2.49	1.38	0.70	8.94
	60.56	2.00	0.52	2.55	2.13	0.46	14.42
	77.18	1.69	0.64	2.86	1.04	0.52	12.19
	75.35	0.72	0.84	2.55	2.18	0.44	14.06
	68.24	2.01	0.67	2.28	1.51	0.85	11.50
	61.42	3.78	0.69	2.07	1.59	0.63	12.67
Mean	68.56	1.94	0.68	2.47	1.64	0.60	12.29
Mature Rat[e]	59.89	1.12	0.72	1.66	1.06	0.62	25.98
	64.00	2.23	0.55	1.29	1.19	0.28	23.92
	63.02	0.67	0.77	1.87	0.70	0.78	28.31
	51.60	0.93	1.19	1.16	0.57	0.86	33.42
	50.93	1.02	0.61	2.08	0.77	0.60	29.45
	50.02	0.99	1.61	1.02	0.47	0.63	28.74
Mean	56.57	1.16	0.91	1.51	0.79	0.62	28.30

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Average weight of animals 102 gm.
e. Average weight of animals 290 gm.

TABLE VI

Effect of a Rachitogenic Diet on the Distribution and Excretion of Cerium-141 in the Rat[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Control Animals[d]	75.42	3.21	0.54	1.54	0.94	0.72	12.93
	56.42	1.00	0.67	1.71	1.18	0.76	15.46
	61.42	2.01	0.64	2.08	1.59	0.63	15.67
	68.24	1.69	0.67	2.28	1.51	0.85	11.50
Mean	65.38	1.90	0.63	1.90	1.30	0.74	13.89
Test Animals[e]	66.48	2.56	0.38	4.27	2.57	0.84	6.17
	59.15	1.63	0.62	4.20	2.86	1.01	5.74
	65.16	1.25	0.74	2.87	2.02	-	9.53
	71.19	2.29	0.48	4.57	2.67	0.68	6.33
	63.67	1.87	1.16	4.19	3.62	0.89	6.56
Mean	65.13	1.92	0.68	4.02	2.75	0.85	6.87

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Average weight of animals 115 gm.
e. Average weight of animals 110 gm.
Rats were maintained on rachitogenic diet for 32 days.

TABLE VII

Effect of Phosphorous-Low Diet on the Distribution and Excretion of Cerium-141 in the Rat[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Control Animals[d]	58.95	1.12	0.93	2.03	1.91	0.16	22.83
	63.02	0.67	0.77	1.87	0.70	0.78	24.31
	59.60	0.93	1.19	1.56	0.57	0.86	23.42
	65.40	1.02	0.61	2.18	0.97	0.60	21.45
Mean	61.74	0.93	0.87	1.91	1.03	0.82	23.00
Test Animals[e]	51.96	1.88	0.73	5.46	2.71	0.16	18.60
	52.93	1.04	1.32	6.38	3.73	0.28	9.89
	60.54	1.88	0.54	4.29	2.27	0.24	17.74
	64.36	1.43	0.56	4.58	3.78	0.47	13.21
	60.83	1.75	0.46	4.14	2.15	0.18	16.92
	61.53	1.79	0.96	5.29	3.35	-	16.77
Mean	58.69	1.64	0.76	5.02	3.00	0.27	15.52

a. Animals were sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Average weight of animals 158 gm.
e. Average weight of animals 80 gm.
Rats were maintained on phosphorous-low diet for 32 days.

TABLE VIII

Effect of Calcium Deficiency on the Distribution and Excretion of Cerium-141 in the Rat[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Control Animals[d]	52.01	1.52	0.56	2.05	0.95	0.28	29.89
	69.01	0.33	0.64	2.24	1.08	1.09	23.85
	59.95	1.82	1.11	1.99	1.10	0.12	28.32
	54.84	0.62	0.94	1.89	1.22	0.68	24.94
Mean	58.95	1.07	0.81	2.04	1.09	0.54	26.75
Test Animals[e]	61.14	0.86	1.29	3.09	1.81	0.27	22.09
	53.62	1.20	1.59	3.29	1.93	0.16	16.38
	60.93	1.36	0.42	3.52	1.76	0.64	26.05
	55.31	1.62	1.13	3.56	2.19	0.33	22.67
	52.59	1.42	1.37	3.05	2.08	0.54	18.91
	58.09	1.29	1.19	2.65	1.45	0.45	18.61
Mean	56.95	1.29	1.16	3.19	1.87	0.40	20.78

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Average weight of animals 175 gm.
e. Average weight of animals 155 gm.
Rats were maintained on calcium-deficient diet for 40 days.

TABLE IX

Effect of Vitamin A-Deficient Diet on the Distribution and Excretion of Cerium-141 in the Rat[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Control Animals[d]	60.82	0.92	0.96	1.74	0.63	0.71	21.64
	53.52	0.99	1.61	1.62	0.87	0.63	24.77
	54.03	0.94	0.86	2.60	1.16	0.78	30.62
	51.17	1.51	0.92	2.05	0.83	0.50	26.75
	55.08	1.08	1.42	1.92	0.96	0.60	20.92
Mean	54.92	1.09	1.15	1.97	0.89	0.64	24.94
Test Animals[e]	64.49	1.62	2.19	2.87	1.17	0.48	11.19
	70.94	1.10	1.58	2.43	1.01	0.92	4.44
	66.03	1.15	1.18	3.10	1.50	0.63	6.84
	57.90	1.13	1.72	3.39	1.58	0.97	8.03
	66.38	1.45	1.26	2.67	1.28	0.83	5.93
	74.10	1.32	0.86	2.38	1.00	0.51	7.84
	67.92	1.75	1.09	2.58	0.99	0.83	9.95
Mean	66.82	1.36	1.41	2.78	1.22	0.74	7.75

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Average weight of animals 205 gm.
e. Average weight of animals 180 gm.
Rats were maintained on Vitamin A-deficient diet for 35 days.

TABLE X

Effect of Calcium-EDTA on the Distribution and Excretion of Cerium-141 in Normal Rats[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Control Animals[d]	52.01	1.52	0.56	2.05	0.95	0.28	29.89
	69.01	0.33	0.64	2.24	1.08	1.09	23.85
	59.95	1.83	1.11	1.99	1.10	0.12	28.32
	54.84	0.62	0.94	1.89	1.22	0.68	24.94
Mean	58.95	1.07	0.81	2.04	1.09	0.54	26.75
Test Animals[e]	58.43	1.31	1.20	1.64	0.69	2.18	26.01
	53.55	1.58	0.98	2.10	1.39	2.32	25.13
	50.55	2.71	0.93	2.16	1.00	1.77	27.41
	45.89	0.76	1.27	2.95	0.84	2.44	31.41
Mean	52.11	1.59	1.09	2.21	0.98	2.17	27.49

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Average weight of animals 175 gm.
e. Average weight of animals 170 gm. Animals injected i.p. with 0.25 mg. calcium-EDTA daily, starting 24 hours after cerium-141 administration.

TABLE XI

Effect of Calcium-EDTA on the Distribution and Excretion of Cerium-141 in Calcium-Deficient Rats[a]

	Whole Liver[b]	Whole Spleen[b]	Kidneys[b]	Bone[c]		Cumulative Excretion[b]	
				Epiphysis	Diaphysis	Urine	Feces and G.I.T.
Control Animals[d]	61.14	0.86	1.29	3.09	1.81	0.27	22.09
	53.62	1.20	1.59	3.29	1.93	0.16	16.38
	60.93	1.36	0.42	3.52	1.76	0.64	26.05
	55.31	1.62	1.13	3.56	2.19	0.33	22.67
	52.59	1.42	1.37	3.05	2.08	0.54	18.91
	58.09	1.29	1.19	2.65	1.45	0.45	18.61
Mean	56.95	1.29	1.16	3.19	1.87	0.40	20.78
Test Animals[e]	45.04	0.80	1.22	3.11	1.82	2.58	31.11
	50.19	1.64	1.38	2.83	1.55	2.73	20.56
	60.34	1.07	1.51	2.76	1.79	2.05	18.67
	51.15	0.62	1.25	3.23	1.46	3.46	24.58
	50.34	1.54	1.08	2.47	1.74	2.22	23.75
Mean	51.41	1.13	1.29	2.88	1.67	2.61	23.73

a. Animals sacrificed 5 days after i.v. injection of 15 μc/kg. cerium-141.
b. Results expressed as per cent of injected dose per organ.
c. Results expressed as per cent of injected dose per gram of fresh bone.
d. Average weight of animals 155 gm. Control and test rats were maintained on a calcium-deficient diet for 40 days.
e. Average weight of animals 165 gm. Animals injected i.p. with 0.25 mg. calcium-EDTA daily, starting 24 hours after cerium-141 administration.

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